# Exploring the Relationship between Soluble Fiber Intake and Bone Mineral Density in Endurance Athletes

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## DEDICATIONS

I wish to dedicate this thesis to my remarkable family and friends. Reflecting on everything I have learned over the past two years has made me realize how truly blessed I am to have an incredible support network. This document would not exist if it were not for the countless words of encouragement and countless hours of assistance in all forms that I received over the past two years. Out of everything that I learned in graduate school, learning how fortunate I am to have their support has been, by far, my most profound lesson. I also wish to dedicate this thesis to Dr. Stella Volpe for the tremendous impact she has had on my life. I am deeply thankful that our life paths intersected, and I will never be able to thank her enough for everything she has done for me.

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# ABSTRACT

Exploring the Relationship between Soluble Fiber Intake and
Bone Mineral Density in Endurance Athletes

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**Background:** Dietary recommendations to athletes are tailored to enhance performance and accelerate recovery. Recommendations focused on these parameters are rarely made in consideration of gut or bone health. Fermentable fibers may play a role in attenuating exercise-induced inflammation and in enhancing calcium absorption; thus, augmenting bone mineral density (BMD) in endurance athletes.

**Objective:** The purpose of this study was to determine whether a relationship could be identified between: 1) soluble fiber and anterior-posterior (AP) lumbar spine BMD among a population of endurance athletes, and 2) total fiber intake and AP lumbar spine BMD among a population of endurance athletes. The study also aimed to assess whether calcium intake, sex, and physical activity levels would influence the relationship between soluble fiber intake and AP lumbar spine BMD.

**Design:** This cross-sectional study included 95 athletes who self-defined their primary form of physical activity as an endurance sport. Dual-energy x-ray absorptiometry scans were employed to measure AP lumbar spine BMD and body composition. A self-administered 2005 Block Food Frequency Questionnaire was provided to assess average daily consumption of macronutrients and micronutrients. Reported energy intake was used to compute an individualized Adequate Intake (AI) for total fiber for each participant, based on the Food and Nutrition Board's AI recommendation that men and women consume 14 grams of fiber per 1,000 kilocalories of energy. Physical activity habits were measured by Actical™ accelerometers, which were worn by a subset of 55 participants for seven days. The accelerometers measured average number of minutes spent in sedentary behavior and light, moderate, and vigorous physical activity states. To determine whether physical activity influenced the relationship between soluble fiber intake and AP lumbar spine BMD, the median time spent in combined moderate and vigorous physical activity was used to divide the population into lower and upper quartile groups, and analyses were conducted in each group and compared. To determine whether sex, meeting fiber AI recommendations, or meeting calcium Recommended Dietary Allowances (RDAs), analyses were conducted in each group and compared.

**Results:** Data were analyzed for 95 athletes (n = 47 male athletes, 48 female athletes; 38.15 ± 10.07 years of age). Twenty-one of the 95 participants met nutrient needs for total fiber (22%), 46 participants met nutrient needs for calcium (48%), and 11 participants met nutrient needs for vitamin D (12%). Fat-free mass, body mass index, and dietary vitamin D intake were significantly correlated with AP spine lumbar BMD ($p = 0.008$, $p = 0.007$, $p = 0.015$, respectively). No significant correlations were found between soluble fiber and AP lumbar spine BMD, or between total fiber intake and AP lumbar spine BMD in the overall sample or among any of the subgroups ($p > 0.05$). No significant between-group differences in regression slopes were found between men and women, between participants who met or did not meet fiber AI guidelines, between participants who met or did not meet calcium RDAs, or between participants in the lower versus the upper quartiles of combined time spent in moderate and vigorous physical activity.

**Conclusions** A majority of participants in this cohort did not meet AI recommendation guidelines for fiber consumption. Nutrient recommendations to athletes may consider encouraging increased fiber consumption to help resolve this gap. The data presented here seem to indicate that fiber intake and bone mineral density are not related. A longitudinal study is required to assess if fiber intake plays a role in bone health in athletes.

# CHAPTER 1: INTRODUCTION

## 1.1 Introduction

Dietary recommendations to athletes are tailored to enhance performance and accelerate recovery. Recommendations focused on these parameters are rarely made in consideration of gut or bone health. It is the position of both the American College of Sports Medicine (ACSM) and the Academy of Nutrition and Dietetics (AND) that athletes should consume low-fiber foods in preparation of physical activity to prevent gastrointestinal complications and ensure rapid delivery of carbohydrate during activity.[1] The current body of nutrition recommendations for athletes does not emphasize fiber consumption during other times, outside of blanket recommendations to follow a healthy eating pattern.[2]

A growing body of literature suggests that fiber may play important roles in: enhancing mineral absorption, attenuating intestinal permeability, reducing lipopolysaccharide (LPS) translocation, and modulating the immune system via fermentation by the microbiota into bioactive short-chain fatty acids (SCFAs).[3-5] These mechanisms have important implications for bone health, and diets rich in fiber may contribute to increased bone mineral density.[6] A 2016 systematic review and position statement of the National Osteoporosis Foundation explained that increased consumption of fibers fermentable to SCFAs has been

positively associated with calcium absorption.[7] However, only one study reviewed by the authors was of long enough duration to measure changes in bone mineral content (BMC) and bone mineral density (BMD); thus, the overall grade of evidence assigned to fiber by the reviewers was C: limited. The researchers reported significant increases in BMC and BMD following a one-year supplementation of 8 g/day fructo-oligosaccharides.[8] More studies are needed to increase researchers' understanding of the effect of fiber on bone health.

The consumption of fermentable fibers may be of increased importance among endurance athletes compared to non-endurance athletes. The physical stress and long duration of endurance exercise, reduced blood flow to the gut during exercise, potential dehydration, and increased body temperature have been shown to increase intestinal permeability.[9-11] These factors have been shown to result in increased translocation of LPS and mild endotoxemia among endurance athletes.[11,12] Other physiological changes during exercise that play a role in increased inflammation include: activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent cascade of stress hormones and cytokines, as well as increased reactive oxygen species (ROS).[2] Researchers have demonstrated that the pro-inflammatory milieu during and immediately following strenuous physical activity stimulates bone resorption biomarkers.[13] Although the body possesses elegant anti-inflammatory feedback mechanisms

that attenuate these cascades in the hours following exercise, endurance athletes spending long durations of time in training may be at increased risk for excessive bone resorption.

The present study aims to evaluate the relationship between anterior-posterior (AP) lumbar spine BMD and soluble fiber intake in endurance athletes. This study will also evaluate how calcium intake, level of physical activity, and sex may influence the aforementioned relationship, and if meeting Adequate Intake (AI) guidelines for total fiber intake significantly contributes to BMD. Therefore, the following Specific Aims and Hypotheses will be examined:

**1.2 Specific Aims**

**Specific Aim 1:**

To determine the relationship between soluble fiber intake and anterior-posterior lumbar spine lumbar bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

**Hypothesis for Specific Aim 1:**

It is hypothesized that there will be a significant positive relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes.

**Specific Aim 2:**

To determine the combined effects of calcium and soluble fiber intakes on anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

**Hypothesis for Specific Aim 2:**

It is hypothesized that higher combined calcium and soluble fiber intake will have a greater positive influence on anterior-posterior lumbar spine bone mineral density compared to participants with lower intakes of these nutrients.

**Specific Aim 3:**

To determine the relationship between total fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-

term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

**Hypothesis for Specific Aim 3:**

It is hypothesized that individuals meeting Adequate Intake guidelines for fiber will have higher anterior-posterior lumbar spine bone mineral density compared to individuals who do not meet Adequate Intake guidelines.

**Specific Aim 4:**

To determine if sex influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

**Hypothesis for Specific Aim 4:**

It is hypothesized that the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes will be stronger among male participants compared to female participants.

**Specific Aim 5:**

To determine if time spent in combined moderate and vigorous physical activity influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

**Hypothesis for Specific Aim 5:**

It is hypothesized that the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density will be stronger among participants spending more time in moderate and intense physical activity.

**1.3 Significance**

It is a truth universally acknowledged that physical activity promotes bone health across the lifespan.[14] The American College of Sports Medicine (ACSM) recommends adults engage in moderate to high intensity weight-bearing endurance activities for 30 to 60 minutes three to five times per week, and resistance exercise for 30 to 60 minutes two to three times per week to help preserve bone health.[14] The National Osteoporosis Foundation has assigned a

strong level of evidence (A) to the effect of physical activity and exercise on bone mass and BMD.[7] Mechanical loading and physical activity have been consistently demonstrated to enhance bone strength in randomized controlled trials and longitudinal studies.[7] However, endurance athletes may not be engaged in the types of activities that maximize the dynamic, high magnitude, short duration, or high impact movements that have been reported by researchers to be most osteogenic.

Endurance athletes engaged in activities such as running, cycling, swimming, and other not weight-bearing sports tend to have lower BMD) than both athletes engaged in weight-bearing sports and individuals who are not physically active.[15] Researchers consistently report that, although runners have higher BMD at primary impact sites (such as the calcaneus and tibia), overall BMD is lower than athletes competing in sprinting, gymnastics, and ball sports.[16] In a cross-sectional study of elite Kenyan runners, Tam and colleagues reported that six of 15 participants had lumbar spine BMD z-scores below -2.0 standard deviations.[17] A systematic review by Olmedillas and colleagues revealed that competitive road cyclists had lower BMD than other athletes and controls, regardless of age and sex.[18]

Reduced BMD across these samples of endurance athletes indicates a need for researchers to elucidate factors driving the discrepancy between the known

benefits of exercise and the cost of prolonged endurance exercise to bone health. The potential for fermentable fiber to promote bone health and perhaps attenuate some of the risks associated with prolonged endurance training is intriguing. An extensive number of animal models, *in vitro* experiments, and supplementation trials in adolescents have demonstrated the role of fermentable fiber and SCFAs in augmenting bone health.[19-22] Attempting to translate these findings into a diverse population of healthy adult endurance athletes is another important aspect of this project.

Researchers have reported that increased fiber consumption correlates negatively with BMD among female athletes.[23,24] However, these studies were conducted on participants with oligomenorrhea and amenorrhea; participants with eumenorrhea either comprised a small percent of the samples or were excluded entirely. Sex steroid deficiency, such that as measured in amenorrhea and oligomenorrhea, induces trabecular bone loss by increasing the lifespan of osteoclasts and reducing the lifespan of osteoblasts.[25] Furthermore, estrogen exhibits antioxidant properties that protect bone, and this protection is lost during periods of low estrogen.[25] The conclusions drawn from these studies should be reevaluated in a healthy population. Few researchers have investigated dietary intake variables influencing BMD among healthy, eumenorrheic female athletes. Furthermore, to the author's knowledge,

researchers have yet to explore the relationship between soluble fiber intake and bone mineral density among healthy adult male and female athletes.

**1.4 Rationale**

**1. Why examine the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density among endurance athletes?**

Endurance athletes undergoing intense training regimens have been shown to exhibit significantly lower bone mineral density than individuals engaged in weight-bearing exercise or individuals who are physically inactive.[15,16] Nutrition guidelines for athletes are designed to optimize athletic performance[1], but they may not be optimized for bone health. Although optimizing performance is certainly a matter of paramount importance to competitive athletes, identifying dietary components that contribute to long-term health and wellness should be incorporated into nutrition recommendations to ensure that training does not pose a detriment to overall health. Examining the relationship between soluble fiber intake and bone mineral density will contribute to researchers' understanding of dietary intake variables that enhance bone health, for both athletes as well as the general population.

Soluble fiber was chosen because soluble fibers have been shown to produce greater concentrations of total short-chain fatty acid concentrations than insoluble fibers in the large intestine.[20] Although both soluble and insoluble

fibers are fermentable, the increased concentrations of SCFA found in response to soluble fiber supplementation indicates that soluble fibers produce more bioactive metabolites. Thus, soluble fiber intake is expected to have a stronger relationship to BMD than total fiber intake.

The anterior-posterior (AP) lumbar spine (L2 to L4) was chosen as the site at which to assess bone mineral density for three reasons. First, the best clinical measures of bone mineral density are dual femoral neck and lumbar spine measures because BMD measurements taken from these sites can be interpreted using World Health Organization (WHO) T-scores.[26] A T-score is calculated by subtracting the mean BMD for healthy young adults (matched to the participant's sex and ethnicity) from the participant's measured BMD and dividing it by the healthy young adult population's standard deviation.[26] A T-score between -1.0 and -2.5 indicates osteopenia, and a T-score below -2.5 indicates osteoporosis. Thus, the dual femoral neck and lumbar spine BMD measures are diagnostically important. Second, the lumbar spine is high in trabecular bone tissue.[27] Trabecular bone contributes structural strength to bone: it is the rods and plates within a sponge-like structure.[7] Conversely, cortical bone tissue is the compact outer shell that protects the trabecular bone tissue and bone marrow.[7] Trabecular bone tissue is more metabolically active than cortical bone, and hence is subject to higher rates of bone turnover.[27] This is another

reason why measuring sites high in trabecular bone is more clinically relevant than measuring sites high in cortical bone or measuring total body BMD. The third reason for selecting lumbar spine BMD stems from the concern that dual femoral neck BMD may be confounded by the varying degrees of impact, compressive force, and shear force in different endurance sports (such as running versus cycling).

Researchers have reported that increased soluble fiber intake may augment bone health, based on findings from animal studies and supplementation trials in adolescents. Replicating the results of these studies in healthy adults remains a gap in the literature. The results of the present study may help to fill this gap.

**2. Why examine the combined effects of calcium and soluble fiber intakes on anterior-posterior lumbar spine bone mineral density among endurance athletes?**

There is a growing body of research in both animals and humans that demonstrates that soluble fiber supplementation enhances the absorption of calcium and other minerals.[8,19-22,28] Researchers hypothesize that one of the mechanisms by which soluble fiber intake is beneficial for BMD is enhanced mineral absorption and the subsequent increased mineral availability for bone accrual. Therefore, it is of interest to assess the combined effects of calcium and

soluble fiber on bone mineral density. Furthermore, inadequate calcium intake may negate any benefit of added soluble fiber consumption, if such a benefit exists. Assessing the combined effects of calcium and soluble fiber intake will facilitate a clearer understanding of the relationship between soluble fiber intake and BMD.

**3. Why examine the relationship between total fiber intake and anterior-posterior lumbar spine bone mineral density among endurance athletes?**

The rationale for studying the relationship between total fiber intake and bone mineral density is threefold. The first purpose is to determine whether meeting Adequate Intake (AI) guidelines contributes in a meaningful way to bone health. Although a distinction is made on nutrition labels, AI guidelines for total fiber do not distinguish between soluble and insoluble fiber, and it is clinically relevant to explore the potential benefits of meeting AI total fiber guidelines.[29] It is also pertinent to explore whether there is a difference between soluble fiber intake (as explored in Specific Aim 1) or total fiber intake and their respective correlations with bone mineral density. The AI guidelines were established based on the median intake of fiber, as a proportion of energy intake, that were shown by researchers to correlate with lowest risk of coronary disease.[29] Insufficient evidence exists to set fiber AI guidelines based on other parameters.[29] Therefore, one of the goals of this project is to determine whether a

total fiber intake conferring a benefit to bone mineral density could be suggested from the present dataset.

**4. Why determine if sex influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density among endurance athletes?**

Processes regulating bone tissue exhibit sex-specific differences across the lifespan. Researchers have shown sex-specific differences in bone mineral accretion rates during adolescence[7] and bone resorption rates as the body ages.[30] Estrogen has a profound effect on bone. Estrogen increases soluble decoy receptor osteoprotegerin, which inhibits osteoclast formation by binding receptor activator of nuclear factor kappa-B ligand (RANKL).[30] Osteoclast differentiation is also inhibited by estrogen blocking macrophage colony stimulating factor. Estrogen stimulates osteoclast precursor cell apoptosis and decreases osteoblast, T cell, and B cell production of RANKL. Although both sexes incur age-related reduced estrogen concentrations and increased rates of bone loss, the acute reduction of serum estrogen concentrations during menopause drastically increases the rate of bone resorption among women compared to men.[30] Examining sex differences in the present study is a necessary tribute to the sex-specific nuances of bone homeostasis.

In a clinically relevant example of sex-specific differences in bone health, Nasiri and Luo compared the risk of hip fracture in a sideways fall between male and female participants, and reported that femoral neck BMD was a better predictor of fracture risk in men than in women. Although femoral neck BMD was significantly correlated with fracture risk for both sexes, the correlation was stronger among male participants ($r = -0.83$, $p < 0.001$) than female participants ($r = -0.68$, $p < 0.001$).[31] Dai and colleagues examined the relationship between fiber intake from fruits and vegetables and bone mineral density in individuals who participated in the Framingham Offspring Study.[32] The researchers reported dietary fiber intake to be significantly associated with decreased rates of bone loss from the femoral neck and trochanter among men, but did not find significant associations among women.[32] These results suggest that there are sex-specific differences in the relationship between fiber intake from fruits and vegetables and bone mineral density.

**5. Why determine if activity level influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density among endurance athletes?**

Although increased physical activity is generally cited in the literature as a strategy for enhancing BMD,[7,14] endurance athletes spending long periods of time in physical activity have been shown to have reduced bone mineral density

compared to other athletes and individuals not engaged in regular physical activity.[16,18,33] The literature remains unclear in terms of quantifying the amount of time spent in endurance activity or quantifying the volume of activity that is needed to elicit an effect on BMD.[34] Examining the relationship between activity level and bone mineral density will help to fill this gap in the literature. It also creates an opportunity to assess if a dietary intake variable (in this case soluble fiber) can attenuate reduced bone mineral density associated with extremely high levels of endurance activity.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Fiber: Definitions, Mechanisms, and Effects

### *2.1.1 Defining Fiber*

Defining fiber is an interesting project. In *Dietary Fiber and Health* (2012), Dennis Gordon quipped: "Dietary fiber is many things to many people. It is a concept, a hypothesis, a marketer's bonanza, a unique complex of non-digestible carbohydrates, but most importantly an integral necessity of a normal functioning and healthy intestine."[35] This quote is a succinct portrayal of the epistemological quandary one finds oneself buried beneath in a quest to define fiber. The meaning shifts from regulatory agency to regulatory agency, from analytical method to analytical method, and from researcher to researcher. This section presents the ways regulatory agencies define fiber, explores the meaning of the term prebiotic, and contextualizes the definitions of fiber in scientific literature.

According to the Institute of Medicine (IOM), total fiber is comprised of dietary fiber and functional fiber.[29] Dietary fiber describes all non-digestible carbohydrates and lignin that are intrinsic and intact in plants, while functional fibers are isolated, non-digestible carbohydrates that confer a beneficial physiological effect in humans.[29] Non-digestible monosaccharides, disaccharides, and sugar alcohols are not included under the auspices of either

definition and are categorized as sugars or sugar alcohols on food labels. Non-digestible animal carbohydrate is excluded from the IOM definition of fiber.[29]

This two-tiered definition of dietary fiber and functional fiber captures the wide range of non-digestible carbohydrates, which may include plant cell walls, storage carbohydrates, and naturally occurring as well as synthesized non-digestible carbohydrates. The flexibility of this definition allows for new types of fiber to be added under the umbrella of "fiber" in the future, while maintaining the ability to provide intake guidelines today. In the United States (U.S.), the Adequate Intake (AI) guidelines for fiber are as follows: 38 grams per day for men and 25 grams per day for women.[29] These guidelines are based on grams of fiber as a proportion of energy intake: 14 grams per 1,000 kilocalories (kcal). The 14 grams per 1,000 kcal guideline is derived from the median intake associated with the lowest risk of coronary artery disease.[36] The IOM states that insufficient evidence exists to establish intake guidelines based on the prevention of colorectal cancer.[29]

The IOM definition represents the way in which United States regulatory agencies define fiber. The Codex Alimentarius Commission is an internationally created set of food standards that is maintained by the World Health Organization (WHO) and Food and Agricultural Organization (FAO). In 2009, the Codex Alimentarius Commission published the definition of fiber as

carbohydrate polymers with at least 10 monomeric units that are not hydrolyzed by human enzymes in the small intestine.[36] This includes edible carbohydrate polymers naturally occurring in foods, isolated carbohydrate polymers, and synthetic carbohydrate polymers. National authorities have the flexibility to optionally include carbohydrates between three and nine monomeric units. Isolated and synthetic polymers must confer a physiological benefit to health, and this benefit must be shown in humans in scientific literature.[36] These health benefits may include improved intestinal transit time, increased stool bulk, fermentation by the microbiota, reduction of serum total or low-density lipoprotein (LDL) cholesterol concentrations, and reduction in post-prandial serum glucose concentrations.[36]

Both the IOM and Codex Alimentarius Commission definitions are similar in that both recognize most types of non-digestible carbohydrates as fiber, regardless of whether the fiber is synthetic. This gives freedom to food scientists and food manufacturers to include added fiber on food labels under "fiber content". The terms "dietary fiber" and "functional fiber" become impossible to tease apart on a nutrition label, however, since the "total fiber" category in the IOM definition is what appears on nutrition labels in the U.S. Another interesting, and pertinent facet of these definitions is the lack of terms prebiotic, soluble, or insoluble. Because of this, there is a disconnect between the ways in

which regulatory agencies define fiber and the ways in which fiber is defined throughout the literature. The regulatory agencies' explanations of fiber may be considered as broad categories that include an array of compounds, more so than they may be considered precise definitions; understanding fiber in this way provides a more useful and meaningful understanding. From the rather broad conceptualization, researchers have narrowed their focus and study one or a small handful of the plethora of compounds that are termed "fiber."

Fiber may be described in the literature as soluble or insoluble. Soluble, or viscous, fibers (such as pectin) dissolve in water to form a gel, while insoluble fibers (such as cellulose) are not miscible with water.[29] Slavin explains in her review that, while the distinction between soluble and insoluble fiber are made on nutrition labels, the scientific evidence distinguishing between the physiological effects of soluble versus insoluble fiber is inconsistent. Soluble fiber has been traditionally associated with reduced cholesterol and postprandial serum glucose concentrations, while insoluble fiber has been associated with increased stool weight and laxation.[29,37]

However, Slavin states that not all soluble fibers have been shown to reduce cholesterol (inulin is one), while some soluble fibers have been shown to increase stool weight and laxation (such as oat bran and psyllium).[37] McRorie et al. suggest that the viscosity of the fiber (high or low viscosity) is a more

important consideration than whether fiber is simply soluble or insoluble.[38] In 2001, the IOM Fiber Panel recommended that the dichotomy between soluble and insoluble fiber be discarded because solubility versus insolubility of a fiber was not a significant predictor of the physiological effects of a fiber.[39]

Indeed, researchers have indicated that a binary classification of soluble versus insoluble fiber eclipses the nuances within each category. These nuances persist in terms of fermentability. Weaver and colleagues tested the effects of six soluble fibers and two insoluble fibers in Sprague-Dawley rats, and reported varying results among six soluble fibers.[20] The study is described in detail in a later section of this review; presently, the key take-away is that, despite the differences among the soluble fibers, all six soluble fibers significantly increased cecal content of short-chain fatty acids (SCFAs), while neither of the two insoluble fibers significantly elevated SCFA content above cellulose controls.

Prebiotics are another term used to describe certain fibers described throughout the literature. First articulated by Gibson and Roberfroid in 1995, the term "prebiotic" was introduced to denote any: "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health."[40] Since this original articulation, the International Scientific Association for Probiotics and Prebiotics (ISAPP) asserted a new definition in

2008; however, the underlying qualification that a prebiotic consists of a fermentable compound exerting a health benefit via modulation by the microbiome did not change.[37] The Food and Agricultural Organization published a definition of prebiotics as follows: "A nonviable food component that confers a health benefit on the host associated with modulation of the microbiota."[41] Part of the guidelines for evaluating a prebiotic requires at least one double-blind, randomized, controlled human trial that provides significant correlation between the proposed benefit/physiological outcome and the modulation of the microbiome.[41]

The European Food Safety Authority (EFSA) uses the FAO definition of prebiotics, and further asserts that a mere shift in the microbiota does not qualify as a "health benefit" (although "health benefit" is generally poorly-defined, and hence, promoting such a claim is rife with caveats).[37] The Food and Drug Administration (FDA) and National Center for Complementary and Integrative Health follow the original Gibson and Roberfroid 1995 definition. In addition, the FDA qualifies prebiotics as Generally Recognized as Safe (GRAS).[37] While regulatory agencies such as the FDA and EFSA are involved in managing health claims and consumer safety of products marketed as prebiotics, the term prebiotics has not filtered into the IOM or Codex Alimentarius Commission definitions of fiber.

Additionally, prebiotics are not labelled as such on nutrition labels. Nutrition labels reflect combined dietary and functional fiber content in foods.[42] Slavin states: "although all prebiotics are fiber, not all fiber is prebiotic."[37] This metaphor reflects the separate impositions placed upon what qualifies as a fiber and what qualifies as a prebiotic. For a specific fiber to be deemed prebiotic, a high level of evidence is required from an RCT. This can be problematic because, although costs have tremendously decreased in recent years, the 16S ribosomal ribonucleic acid (16S rRNA) or phylogenetic sequencing required to quantify shifts in the microbiome are expensive.

Furthermore, the stipulation that a microbial population shift does not qualify as a health benefit does not prevent researchers from reporting correlations between population shifts and an outcome variable as evidence that a benefit could be identified.[43] A major limitation of microbiome research is the need for reproducible results,[44] and the narrative of industry regulation of prebiotics is rife with retractions.[45] Food scientists are motivated to study the potential health benefits of prebiotic fibers in the interest of marketing food products. Consequently, the term prebiotics has seeped into scientific discourse, and the term is unavoidable in the literature of fiber research. It is important to be aware of these caveats when reviewing the literature because the conclusions of each study must be carefully and individually evaluated.

*2.1.2 Fiber Fermentation: Mechanisms*

The human body is a habitat to trillions of microorganisms. These microorganisms have carved adaptive ecosystems with diverse taxonomic profiles across the geography of the body; these ecosystems shift in response to fluctuations in host health, age, diet, location, and so on *ad infinitum*.[46] Researchers have established that (by conservative estimates) hundreds of unique species of microorganisms live in the gastrointestinal tract, and the strains of bacteria within the gut microbiome are individualized and distinct.[47] These microorganisms also share distinct gene pools that vary by substrate availability in the gut (i.e., the composition of the host's diet), and gene selection is driven by environmental selection.[48] The relationship between host diet and the species composition of the microbiome is complex; increased substrate availability contributes to increased fermentation product concentrations. These fermentation products reduce colonic pH and increase the availability of cross-feeding metabolites, further altering species composition.[49]

A detailed description of the taxonomic and phylogenetic composition of the gut microbiome is not the purpose of the current project. "Gut microbiome" is a term that, at best, describes a diverse series of fluctuations spanning time, space, and individuals. The important take-aways from the vast field of microbiome literature are: microbiomes metabolize available substrates into an

array of bioactive substances that influence host health, increased diversity is generally (albeit not always) associated with improved health of the microbiome, and there is no set standard for what comprises a "healthy" microbiome because researchers consistently find compositional differences among healthy individuals.[46,47,50] The focus of the current project is not to dwell on these nuances, but rather to elucidate the overarching ways fiber has been shown to influence bone health via a core set of metabolic pathways that have been identified across phyla.

The primary metabolic pathways of interest are the fermentation of fibers into short-chain fatty acids (SCFAs). The microbiome contains genes that hydrolyze the glycosidic bonds linking the monosaccharide molecules on a chain of fiber. Humans do not produce these enzymes, which is why fiber fermentation is unique to the microbiome. Once the glycosidic bonds are hydrolyzed, monosaccharides are freed into the luminal space and may be reduced to phosphoenolpyruvate (PEP).[51] Acetate, proprionate, and butyrate are the three major SCFAs that are produced along four distinct fermentation pathways beginning with PEP and ending with the SCFAs.[51]

den Besten and colleagues explain that one of the mechanisms by which fibers are beneficial to human health is the reduction of colonic pH by the presence of SCFAs. When pH in the distal colon exceeds 6.5, as is measured

during limited dietary fiber intake, the population of butyrate-producing bacteria declines and the population of acetate and propionate producing bacteria increases. Colonocytes prefer butyrate as an energy source.[51] Butyrate also acts as a histone deacetylase (HDAC) inhibitor, upregulating the expression of proregenerative genes and downregulating genes associated with colon cancer.[52] Another important role of butyrate is anti-inflammatory signaling through G protein-coupled receptors (GPCRs).[52] This could be of particular importance among endurance athletes, because exercise increases inflammation at the site of the intestinal barrier and diminishes tight junction protein integrity.

*2.1.3 Fiber Fermentation: Effects on Bone Health*

Researchers have investigated the effects of fiber on bone mineral density in a variety of contexts. Animal models have shown positive effects of fiber supplementation on BMD. These results have been replicated in humans in clinical trials of fiber supplementation in adolescent boys and girls.

To compare the effects of eight different types of fibers on bone health, Weaver and colleagues measured calcium absorption, mineral retention, bone mineral content, and cecal SCFA concentrations in a 12-week fiber supplementation trial among 150 male Sprague-Dawley rats.[20] The rats were separated into 10 groups: eight groups received a different test fiber supplementation, and the remaining two groups received a cellulose control

fiber supplementation. The eight test fibers included: two types of insoluble resistant starch, soluble corn fiber (SCF), soluble fiber dextrin (SFD), soluble pullulan, a soluble polydextrose (PDX), inulin, and a combination of inulin and short-chain fructo-oligosaccharide (FOS). The fiber supplementation initially replaced 10% of the cornstarch in a standard AIN93 G diet formula, but loose stools in several groups led the researchers to reduce the fiber content to 5% two weeks into the intervention. After another three weeks, the fiber content in the SCF, SFD, and PDX groups were reduced to 4% test fiber and 1% cellulose due to continued loose stools.[20] The high prevalence of side effects and inconsistency among the groups are two limitations of this study.

Calcium absorption was measured by a calcium radioisotope ($^{45}Ca$) absorption test. Rats were fed a 5 gram test meal of their assigned diet with 10 microcuries ($\mu$Ci) of $^{45}Ca$ two days prior to sacrifice. Forty-eight hour $^{45}Ca$ uptake in femurs was measured by a liquid scintillation counter. Femur $^{45}Ca$ uptake was significantly higher than controls in the inulin/FOS group ($p < 0.05$), but not significantly higher in any other group. Mineral retention was determined by subtracting losses in urine and feces from total intake. Calcium retention did not significantly differ from controls in any of the fiber groups. Zinc retention was significantly higher in the insoluble resistant starch groups, the SFD group, and the inulin/FOS group ($p < 0.05$). Magnesium retention was significantly higher in

the SFD group, and copper retention was significantly higher than controls in all groups except for inulin ($p < 0.05$).[20] These data suggest that different fibers influence the retention of minerals in unique ways.

Bone mineral density (BMD) and bone mineral content (BMC) were measured by peripheral quantitative computed tomography (pQCT) and inductively coupled plasma atomic emission spectroscopy (ICP-AES), respectively. Rats in the soluble corn fiber group and soluble fiber dextrin group had significantly higher BMD and BMC than the control groups ($p < 0.05$). None of the other types of fibers were significantly associated with higher BMD or BMC compared to the control group. Bone breaking strength was assessed by three point bending. Among all groups, SCF and SFD were the only two groups reflecting significant increases in resistance to fracture.[20] These findings demonstrate a clinically relevant association between the intake of SCF and SFD and reduced fracture risk, and suggest the need for research of these fibers in humans to evaluate whether results are translatable to a human population.

Weaver et al.'s[20] study is an important contribution to the literature on fiber and bone health for two major reasons. Foremost, it demonstrated that not all fibers elicit the same physiological effects. Second, this study set the stage for future research by isolating two fibers (SCF and SFD) as having clinically relevant implications. By conducting their study on rats, the authors conducted

a tightly controlled experiment on a wide variety of fibers to tease out the differences among them before conducting research on fibers among human participants. Two studies were published after Weaver et al.'s[20] study, that utilized SCF as the intervention fiber.

Whisner and colleagues evaluated the effects of soluble corn fiber (SCF) supplements on calcium absorption, bone biomarkers, and microbiome population composition among 24 adolescents, 12 to 15 years of age (9 female participants and 15 male participants). In this double-blind, cross-over design, participants were randomized into either a treatment group with a total of 12 grams of SCF supplemented in fruit snacks (6 grams each at lunch and dinner), or a control group with zero grams of SCF supplement. A seven-day washout period occurred between group assignments. Participants were housed at Purdue University throughout the duration of the study, and were assigned a standard diet containing approximately 15 grams of fiber and 600 milligrams of calcium. Energy needs were determined using the Harris-Benedict equation.

The researchers measured fractional calcium absorption via $^{44}Ca$ and $^{43}Ca$, two stable calcium isotopes administered orally with a control meal and intravenously one hour after the control meal, respectively. Two 24-hour urine pools were collected from each participant spanning the 48 hours after the calcium isotope administration. Although mean fractional calcium absorption

did not differ between groups over the first 24 hours, there was a significant increase (11.6%) in calcium absorption 24 hours to 48 hours following SCF supplementation ($p = 0.02$). These findings support the hypothesis that changes in calcium absorption may be related to shifts in microbial population composition, since the delay in increased absorption may be related to the time needed to induce population shifts. Despite this significant increase in fractional calcium absorption, the net calcium absorption efficiency did not differ between the two groups. Additionally, calcium retention, urinary calcium concentrations, and fecal calcium concentrations did not significantly differ between the supplementation group and the control group.

Serum alkaline phosphatase, phosphorus, calcium, parathyroid hormone, insulin-like growth factor 1, insulin-like growth factor-binding protein 3, and sclerostin were also measured. No significant differences in the serum concentrations of these biomarkers were reported between groups. Urine concentrations of cross-linked N-telopeptides of type I collagen, calcium, phosphorus, and creatinine were measured, and no significant differences were found between groups in the urine concentrations of any of these biomarkers.

The researchers assessed changes in microbial population composition via 16S ribosomal ribonucleic acid (16S rRNA) sequencing of fecal samples. Phylum-level shifts were noted following both SCF supplementation and control

conditions, with members of the Bacteroidetes phylum significantly increasing ($p < 0.05$) and members of the Firmicutes phylum significantly decreasing ($p < 0.05$). The intervention was shown to have an effect at the family level: following SCF supplementation, significantly increased proportions of bacteria in the Porphyromonadaceae ($p = 0.02$) and Clostridiales families ($p = 0.009$) and significantly decreased proportions of bacteria in the Peptostreptococcaceae family ($p = 0.04$) were reported. At the genera level, proportions of *Enterococcus* ($p < 0.03$), *Anaerofustic* ($p < 0.05$), and *Coprococcus* ($p < 0.03$) were significantly decreased. Despite these changes, overall alpha diversity values did not differ significantly between treatment and control groups. Increased calcium absorption was shown to correlate positively with proportions of *Bacteroides* ($p = 0.027$), *Butyricicoccus* ($p = 0.039$), *Oscillibacter* ($p = 0.008$), and *Dialister* ($p = 0.003$) genera. Increased calcium absorption was shown to correlate negatively with proportions of *Actinomyces* ($p = 0.009$) and *Pseudomonas* ($p = 0.03$) genera.[21]

While these findings are interesting, the researchers did not assess the functional changes to the microbiome; thus, the mechanisms underlying the increase in calcium absorption remain unclear. Although the population shifts demonstrate that fiber may have elicited an effect on the microbiome, it is interesting to note that simply changing the participants' environment by bringing them into the camp setting also induced shifts in the population

composition. These shifts speak to the dynamic array of influencers that researchers have identified as mediators of microbial population composition. More research is needed to assess the effect of fiber on microbial functional gene expression, as well as the impact of environment on microbial functional gene expression. Furthermore, there is a wide gap between improved calcium absorption and enhanced bone mineral density. The authors did note that the short duration of the trial rendered an assessment of bone mineral density impossible. Future research is needed to examine whether increased fiber intake influences bone mineral density in a meaningful way.

In a randomized, cross-over dose-response study, Whisner and colleagues evaluated the effects of three doses of SCF supplements on calcium absorption and fecal microbial community composition in 28 healthy, free-living adolescent female participants.[22] During each arm of the trial, participants were administered muffins and beverages fortified with either 0 grams, 10 grams, or 20 grams per day of SCF for four weeks. Fecal samples were collected during each supplementation arm. In addition, pH, short-chain fatty acid content, and microbial population composition were measured. After each arm of the trial, participants were housed at Purdue University during a three-day clinical visit and fed a controlled diet. Fractionated calcium absorption was measured in 12-hour increments via a dual-stable calcium isotope absorption test. Urine

concentrations of N-telopeptides of collagen crosslinks, a bone resorption marker, were measured. Fasting serum concentrations of bone-specific alkaline phosphatase, osteocalcin, and intact parathyroid hormone were measured to evaluate changes in bone formation and calcium metabolism.

Calcium absorption during the 10 gram SCF supplementation arm increased significantly, by 13.3%, compared to the control arm ($p = 0.042$). Calcium absorption during the 20 gram SCF supplementation arm increased significantly, by 12.9%, compared to the control arm ($p = 0.026$). Additionally, the number of operational taxonomic units (OTUs) increased significantly in both the 10 gram and 20 gram supplementation groups compared to the control group ($p < 0.05$). Shifts at the genus and family levels were also reported. The authors reported a dose-response effect between the 10 gram and 20 gram groups among the genera *Parabacteroides* and *Dorea*, which were significantly increased and decreased, respectively, in the 20 gram dose group compared to the 10 gram dose group ($p < 0.05$). *Bacteroides* and *Lachnospira* were significantly increased in the 20 gram supplementation group but not in the 10 gram supplementation group ($p < 0.05$). No significant differences were reported between groups among bone resorption or calcium metabolism biomarker concentrations. These results[22] concur with the findings of Whisner and colleagues[21], and contribute to the

literature by noting a dose-response effect of SCF on microbial population composition.

Despite Whisner and colleagues' new finding of a dose-response effect of SCF supplementation on the microbiome, the trial[22] was not long enough to assess whether prebiotic consumption exerts any long-term effect on bone mineral density. It is also unclear whether the dose-response changes to the microbiome are clinically relevant to bone health, because no significant dose-response was reported for calcium absorption. More research is needed to determine whether prebiotic consumption contributes to bone mineral density.

A recently published abstract by Dai and colleagues[32] may shed some light on the effect of prebiotic fibers on BMD at later stages of the lifespan.[32] The researchers examined the relationship between fiber intake and bone mineral density in older adults who participated in the Framingham Offspring Study.[32] The researchers reported dietary fiber intake to be significantly associated with decreased rates of bone loss from the femoral neck and trochanter among men over an 8.1-year period; but, did not find significant associations among women.[32] These data suggest that increased fiber intake among men may be protective against bone loss.

Taken together, these three aforementioned studies contribute to researchers' understanding of the role of prebiotics and fiber in adolescents and

older adults. However, there is a lack of research on the relationship between fiber and bone mineral density among healthy adults. While the focus has been examining variables that influence bone accrual during adolescence and bone loss during aging, it is equally clinically relevant to assess variables that may contribute to maintaining bone density during adulthood. This is particularly important to assess among adults who may be at increased risk for bone loss, such as endurance athletes.

## 2.2 The Pathophysiological Effects of Endurance Training on the Gut

### *2.2.1 Introduction*

The prolonged physical activity characteristic of endurance training elicits numerous physiological responses that affect gut and bone health. The purpose of this section is to describe several of these physiological responses and contextualize their effects on the gut and on bone mineral density, so that the benefits of fiber for athletes may be better understood. Exercise represents a challenge to cardiovascular and metabolic homeostasis,[53] and this challenge activates the hypothalamic-pituitary-adrenal (HPA) axis.[9,54,55] The cascade of hormones that are released in response to HPA axis activation have specific consequences for the epithelial cells of the intestines and bone homeostasis. Additionally, reduced blood flow to visceral organs during exercise can result in gastrointestinal ischemia.[56] Hyperthermia, reactive oxygen species (ROS)

production, and dehydration also contribute to the negative effects of endurance training to the intestinal barrier.

*2.2.2 The Physiology of the Gut Barrier*

Researchers are interested in measuring exercise-induced damage to the intestines because gastrointestinal distress, nausea, vomiting, and diarrhea during athletic events compromises performance.[9] The mucosal layer, vascular endothelium, and the epithelial cells lining the gastrointestinal tract comprise a multi-layer structure that is a physical barrier between the body and luminal contents. This structure is also a chemical and immunological barrier of digestive secretions, cytokines, peptides, and immune molecules.[57] Major functions of the gut barrier include regulating the absorption of nutrients and water, and maintaining the coexistence between microorganisms of the gut microbiome and the host.[57] Disease states and impaired absorption of nutrients may result when barrier homeostasis is disrupted because these functions are compromised.

The paracellular space between the single layer of continuous epithelial cells is sealed by tight junctions that regulate the paracellular movement of water and solutes between the lumen and circulation.[11,58] Researchers have identified over 50 proteins that contribute to regulating tight junctions.[9] Among them are a multitude of transmembrane proteins, scaffolding proteins that cluster signal

transduction, enzymes, polarity maintenance proteins, and transcription factors.[58] Zonula occludens (ZO-1, ZO-2, ZO-3) proteins are cytoplasmic scaffolding proteins to which occludin, claudins, and tricellulin bind to link the actin cytoskeleton of adjacent epithelial cells.[57] Although tricullin, occludin, and the recently discovered marvelD3 proteins may be interchanged, severe barrier leakage occurs if all three are down-regulated or disrupted during homeostatic imbalances.[57]

Tight junction proteins regulate the barrier integrity via their phosphorylation state. Phosphorylation of a tight junction protein can either increase or decrease the integrity of the bond between the junction protein and the scaffolding protein.[11] Phosphorylation of occludin, claudin, or ZO-1 by novel protein kinase C (nPKC) reduces barrier permeability by enhancing the interaction between proteins. Phosphorylation of claudins by protein kinase A (PKA) increases permeability by reducing its interaction with ZO1, and phosphorylation of occludin by conventional protein kinase c (cPKC) increases permeability by reducing its interaction with ZO-1.[11] This brief background on the physiology of the gut barrier and the contribution of phosphorylation enzymes to barrier is an important backdrop for evaluating exercise-specific effects on the gut barrier.

*2.2.3 Translocation of Lipopolysaccharide Following Diminished Barrier Integrity*

Diminished barrier integrity creates an environment in which bacterial lipopolysaccharide (LPS) is translocated from the lumen into the bloodstream by both transcellular and paracellular routes.[11,59] Lipopolysaccharides are fragments of cell wall from gram-negative bacteria.[39] When LPS enters circulation, surface receptors cluster of differentiation 14 (CD14) and toll-like receptor 4 (TLR4) on cells of the innate immune system bind LPS, initiating a cascade of pro-inflammatory cytokine secretion.[9,39] Tumor necrosis factor alpha (TNF$\alpha$), interferon-gamma (IFN$\gamma$), and interleukins 1-beta (IL1$\beta$) and 6 (IL-6) are released.[9] Interferon-gamma and TNF$\alpha$ have been shown to increase tight junction openings by contracting the actin skeleton through the activation of myosin light chain kinase and consequent phosphorylation of myosin light chain.[11] Thus, LPS diminishes barrier integrity by altering the phosphorylation state of tight junction proteins.

Researchers have investigated serum concentrations of LPS following strenuous exercise since the late 1980's. Many researchers have demonstrated that LPS concentrations are significantly increased followed long bouts of strenuous exercise.[12,60,61] Lower degrees of intensity and shorter durations of exercise may account for the lack of significant findings among LPS

concentrations by other researchers, despite significant increases in intestinal permeability markers.[62,63]

*2.2.4 Other Exercise-Induced Factors Affecting Intestinal Permeability*

Hyperthermia, reactive oxygen species (ROS) generation, and dehydration are additional variables that increase risk for gastrointestinal injury during exercise.[64,65] King et al. reviewed studies investigating the influence of temperature and fluid balance on ROS production during exercise.[66] Hyperthermia has been shown to increase the formation of ROS, diminish antioxidant defense, and increase intestinal permeability during low-level heat stress in rats.[66] These three factors are believed to occur from splanchnic hypoperfusion during heat stress and increased blood viscosity.[66] King et al. incorporated a variety of protocols in their review of research in humans, and concluded that hyperthermia induces ROS in plasma regardless of exercise protocol or hydration status.[66]

Pires et al. conducted a systematic review of studies where researchers investigated the relationship between core body temperature and intestinal permeability in healthy adults, and reported a strong positive correlation between core temperature and intestinal permeability in most of the 16 studies reviewed.[67] One link between hyperthermia and increased intestinal permeability may be the increased ROS that are generated when body

temperature increases. A mechanism by which ROS increases intestinal permeability may be tyrosine phosphorylation of occludin by hydrogen peroxide.[11] Hydrogen peroxide is one type of ROS. The phosphorylation of tyrosine causes occludin to translocate into the intracellular membrane of epithelial cells, thus diminishing the interaction between occludin and ZO-1 and increasing permeability.[11]

Dehydration has also been shown to contribute to intestinal damage during exercise. Lambert and colleagues measured the intestinal permeability of 11 male and nine female runners with a mean age of 22 ± 3 years and mean maximal oxygen consumption of 55.7 ± 5.0 milliliters of oxygen per kilogram of body weight per minute (mL/kg/min) of exercise.[68] Participants ran for 60 minutes on a treadmill at 70% of their maximal oxygen consumption under each of the following three conditions: running with no fluid, running with ingestion of a 4% glucose solution, and running with ingestion of plain water.[68] A fourth condition in which participants remained at rest for 60 minutes served as a control. Before each of the four conditions, participants consumed a solution containing 5 grams of sucrose, 5 grams of lactulose, and 2 grams of rhamnose. The urinary excretion ratio of lactulose to rhamnose was used as a measure of small intestine permeability, and the urinary excretion of sucrose was used as a measure of gastroduodenal permeability.

The researchers reported that the lactulose to rhamnose ratio was significantly higher among participants running with no fluid (lactulose to rhamnose ratio = 0.0625) compared to the rest condition (lactulose to rhamnose ratio = 0.035) ($p < 0.008$). Gastroduodenal permeability was significantly higher among participants running in the no fluid condition compared to rest ($p < 0.008$). There were no significant differences between the rest condition and either of the other two conditions. Participants lost 1.5% of their body mass in the running with no fluid condition compared to the rest condition, which constituted a significant body mass loss ($p < 0.05$). No significant body mass losses were reported for the other groups. These findings indicate that dehydration may contribute to increased intestinal permeability in runners.

*2.2.5 Exercise and Splanchnic Hypoperfusion*

Exercise requires an immense increase in cardiac output and a redistribution of blood flow into active skeletal muscle tissue.[69] The body compensates for the increased demands to skeletal muscle by redistributing blood flow away from visceral organs and into skeletal muscle tissue. During maximum intensity exercise, splanchnic blood flow can decrease by up to 80%.[70] Splanchnic circulation involves blood flow to the visceral organs from the celiac trunk and the inferior and superior mesenteric arteries, and blood flow from the trunk by the inferior and superior mesenteric veins and portal vein.[71] The release

of norepinephrine by the sympathetic nervous system during strenuous physical activity forces this system into a state of vasoconstriction, resulting in splanchnic hypoperfusion.[71] Vasoconstriction also occurs by non-adrenergic pathways: angiotensin II receptor, type 1 (AT-1) also contributes to the redistribution of blood away from visceral organs during physical activity.[69]

Although splanchnic hypoperfusion is a necessary adaptation that facilitates increased blood flow to skeletal tissue, it has the undesirable possible consequence of inducing ischemia of the epithelial cells of the intestines. This occurs when adenosine triphosphate (ATP) concentrations are depleted from lack of oxygen.[72] Under these circumstances, the tight junctions that maintain barrier function are compromised, and contents from the lumen can leak into interstitial space and enter the bloodstream.

Researchers have investigated whether this reduced blood flow could result in intestinal injury. van Wijck et al.[62] conducted a study on nine healthy male participants with a mean age of 23.6 ± 0.7 years, to investigate the effects of a 60-minute cycling session at 70% of maximum workload capacity on intestinal cell health. Gastric tonometry was used to measure the luminal intragastric partial pressure of carbon dioxide ($pCO_2$). This method allows researchers to distinguish between a change in perfusion and a true oxygen deficit, because ischemic tissues release excess carbon dioxide from bicarbonate ($HCO_3^-$)

buffering during anaerobic respiration.[73] Plasma intestinal fatty acid-binding protein (I-FABP) was used as another indicator of intestinal damage. Since I-FABP is an intracellular protein, it is elevated in plasma only in the case of small intestinal mucosal tissue injury.[74]

van Wijck et al.[62] reported that $pCO_2$ and I-FABP were significantly elevated during exercise.[62] Furthermore, they reported a significant positive correlation between $pCO_2$ and I-FABP ($p < 0.001$). Both measures recovered to baseline concentrations within one hour post-exercise. Biomarkers of liver damage, including liver fatty acid binding protein (L-FABP) and aspartate transaminase (AST) were significantly increased in all participants immediately following exercise ($p < 0.001$), and plasma concentrations of alanine aminotransferase (ALT) were significantly increased immediately following exercise ($p < 0.01$). Alpha-glutathione S-transferase (alpha-GST) was significantly increased one hour post-exercise ($p < 0.01$).[62] These findings suggest that splanchnic hypoperfusion affects the liver as well as the intestines.

In addition to the aforementioned variables, plasma concentrations of myeloperoxidase (MPO) and plasma and fecal calprotectin concentrations were assessed at baseline and immediately after cycling to assess inflammation. Significant increases were observed in MPO ($p < 0.001$), serum concentrations of calprotectin ($p < 0.0001$), and fecal concentrations of calprotectin ($p < 0.05$).[62]

Given the vast array of pro-inflammatory markers that are used to measure inflammation, the authors' choice to measure MPO reflects their interest in measuring intestine-specific damage rather than systemic inflammation. Myeloperoxidase is released by neutrophils during degranulation to generate oxidation products that inhibit microbes.[58] Calprotectin concentrations are usually used to evaluate the progression of inflammatory bowel disease.[75] These proteins measure neutrophil activity specifically within the intestinal mucosa: elevations in these proteins indicate that specific intestinal injury occurs during exercise. Myeloperoxidase concentrations are elevated in individuals infected by *H. pylori*; however, the authors did not explore this as a potential confounding variable.[76]

van Wijck et al.[62] also assessed gastrointestinal permeability among a subset of six participants. Small intestine permeability was measured via lactulose/L-rhamnose ratio in urine and plasma. While urinary lactulose/L-rhamnose ratios were not significantly different, the plasma lactulose/L-rhamnose ratio was significantly increased after exercise ($p < 0.001$).[62] Plasma endotoxin core antibodies were also measured before and after the cycling test, and no significant differences were reported. Interestingly, the researchers measured endotoxin antibodies (immunoglobulin G), rather than directly measuring lipopolysaccharide (LPS) concentrations. Their findings conflict with

results from others who have reported significant increases of serum LPS concentrations among runners.[9,12,61] Although van Wijck et al.'s[62] study had a small sample size and did not include female participants, these results suggest that there is a relationship between splanchnic hypoperfusion and intestinal ischemia.

Another consequence of splanchnic hypoperfusion is the possibility of reperfusion when blood flow returns to normal levels. As oxygen floods ischemic, hypoxic tissues, further injury response occurs from peroxidases, xanthine oxidase, lipoxygenases, glucose oxidase, and several other enzymes.[76,77] When hypoxanthine is reduced to xanthine by xanthine oxidase, hydrogen peroxide is formed and the tight junction proteins are disrupted.[11] Intestinal injury resulting from exercise may be a combination of ischemia with reperfusion, since oxidative stress and inflammation can result when blood flow is restored.[71] *In vivo* animal studies, *in vitro* experiments using Caco-2 cells, and studies in critically ill populations and surgery patients have demonstrated exacerbations from hypoxia/reoxygenation.[77-79] More research is needed, however, to determine whether intestinal injury from reperfusion occurs among healthy athletes. Research has been conducted on ischemia and reperfusion in skeletal muscle tissue in athletes,[80-82] but there are no studies to the author's knowledge regarding ischemia/reperfusion in intestinal tissue in athletes. While

authors of review articles have discussed ischemia/reperfusion as possibly playing a role in intestinal injuries in athletes, they unfortunately extrapolate results from studies conducted in animal models, *in vitro* experiments, critically ill/surgery patients or cite previously published reviews committing this fallacy.[12,71,83] Given the lack of research in healthy adults, this topic will not be explored here.

*2.2.6 The Hypothalamic-Pituitary-Adrenal (HPA) Axis*

The activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis by a stressor initiates a cascade of stress-mediating hormones, including corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol.[58] Exercise has been demonstrated to activate the HPA axis,[84] and the cascade of hormones released by the HPA axis may account for some of the gastrointestinal symptoms resulting from prolonged or intense physical activity. Physical activity that is between a minimum of 50% and 60% of maximal oxygen consumption ($VO_2max$) has been reported as the critical threshold of intensity that is needed before ACTH and cortisol concentrations increase in response to exercise.[9] A variety of compounds induce the paraventricular nucleus to release CRH, and compounds released during exercise that induce the release of CRH include catecholamines, interleukin-1, interleukin-6, and tumor necrosis factor-$\alpha$.[58,85] The HPA axis is a negative feedback loop: pro-inflammatory cytokines trigger the

HPA axis, and anti-inflammatory compounds are then secreted to regulate stress response.[86] In addition to CRH, glucocorticoids (including cortisol) are also released from the adrenal glands. Glucocorticoids are also produced by epithelial cells in response to lipopolysaccharide (LPS) and pro-inflammatory cytokines.[87]

Many tissues contain CRH receptors, and the gastrointestinal tract contains both types of G-protein coupled CRH receptors: corticotropin releasing hormone receptor 1 (CRHR1) and corticotropin releasing hormone receptor 2 (CRHR2).[58,59] While CRHR1 receptors are involved in the basal activities of the HPA axis and are saturated by small concentrations of CRH, CRHR2 receptors mediate the stress response activities of the HPA axis.[58] Researchers have demonstrated in animal and *in vitro* studies that CRH contributes to stress-induced changes to the gastrointestinal tract through both CRHR1 and CRHR2 receptors.[58] These changes include intestinal inflammation, upregulation of CRH expression in colonic lamina propria immune cells, increased colonic motility, increased intestinal permeability, and accelerated colonic transit.[58,87,88]

Rhee et al. conducted an *in vitro* study and demonstrated that CRH increased the expression of vascular endothelial growth factor A (VEGF-A) in human colonic epithelial cells via activation of CRHR1 or CRHR2 receptors.[89] Vascular endothelial growth factor A plays a role in colitis-associated

inflammatory angiogenesis.[89] In a study on 36 healthy, non-smoking volunteers, Pritchard et al. reported that CRH injection significantly constricted the small intestine ($p = 0.003$), significantly increased ascending colon volume ($p = 0.002$), and significantly increased participants' sense of distension ($p = 0.043$).[90] These data demonstrate that increased corticotropin releasing hormone shifts the homeostatic balance of both the small and large intestines.

Bonifazi et al. investigated the effects of training on the messenger ribonucleic acid (mRNA) expression of glucocorticoid receptor-alpha.[91] The authors hypothesized that athletes undergoing daily endurance training may experience higher endogenous cortisol concentrations than individuals not participating in endurance training regimens, and that elevated concentrations of cortisol would correlate negatively with the mRNA expression of glucocorticoid receptor -alpha.[91] The authors measured the glucocorticoid receptor-alpha mRNA expression from peripheral blood mononuclear cells in nine highly trained male swimmers (mean training volume = 21.6 ± 1.7 hours per week, mean age = 25.7 ± 3.5 years), eight low-trained male runners (mean training volume = 6.4 ± 2.6 hours per week, mean age = 29.4 ± 5.3 years), and nine untrained male participants (mean age = 29.0 ± 6.4 years).

Glucocorticoid receptor-alpha mRNA expression was 10 times less in the highly-trained group compared to the untrained group, while glucocorticoid

receptor-alpha mRNA expression was two times less in the low-trained group compared to the untrained group. A one-way analysis of variance was conducted to assess between groups differences, and a significant difference between the three groups was reported ($p < 0.001$). However, the authors did not conduct a post hoc test to determine where these differences occurred. There were no significant differences among groups for adrenocorticotropic hormone and cortisol concentrations, which the researchers expected, because samples were taken when all participants were at rest. The down-regulation of glucocorticoid receptor-alpha in the highly trained and low-trained groups suggests that frequent exposure to stressful stimuli (training) leads to increased exposure to acutely elevated cortisol concentrations.

Nieman et al. reported significantly increased concentrations of serum cortisol in 20 trained male cyclists following a 75 kilometer (km) time trial.[92] Participants' mean age was 38.4 ± 6.0 years and their mean maximal oxygen consumption was 47.9 ± 7.8 mL/kg/min. Serum cortisol concentrations increased from 10.7 ± 4.3 micrograms per deciliter (mcg/dL) to 28.4 ± 10.5 mcg/dL ($p < 0.001$).

Oosthuyse and colleagues measured serum cortisol concentrations as part of their study on bone resorption biomarkers among ten well-trained Caucasian male cyclists. Participants' mean age was 29.6 ± 11.1 years, and all participants

were healthy, non-smokers. Participants reported a mean 8.3 ± 4.6 years of cycling experience and a mean weekly training volume of 12.7 ± 6.2 hours per week.[93] Blood samples were withdrawn before and after a three-hour race-simulated indoor cycling test that was repeated over four consecutive days. On test days one and two, post-exercise plasma cortisol concentrations were significantly increased compared to pre-exercise concentrations ($p < 0.01$). On test day three, post-exercise plasma cortisol concentrations were significantly increased compared to pre-exercise concentrations ($p < 0.05$). On test day four, post-exercise plasma cortisol concentrations were significantly increased compared to pre-exercise concentrations ($p < 0.001$).[93]

Post-exercise cortisol concentrations on day three were significantly lower than post-exercise cortisol concentrations on day 1 ($p < 0.05$), and pre-exercise cortisol concentrations were significantly lower on days two, three, and four ($p < 0.01$) compared to pre-exercise cortisol concentrations on day one.[93] Although the reduced pre-exercise cortisol concentrations reflect an adaptation to the stress of exercise, the exercise test continued to induce significantly increased cortisol concentrations across all four days of testing.

A limitation shared by both of these studies is their inclusion of only male participants. There have not been any published studies, to the author's knowledge, on healthy, pre-menopausal eumenorrheic female athletes where

serum cortisol concentrations were measured. There appears to be a bias in the literature for focusing on the female athlete triad with respect to female athletes, rendering an assessment of the physiology of stress hormones in healthy, eumenorrheic female athletes difficult. Increased cortisol concentrations have been associated with reduced bone mineral density in non-athlete populations. Osella et al. assessed the relationship between cortisol concentrations and measures of bone health in 82 healthy women with onset of menopause between six months and five years before participation in study protocol (mean age = 52.3 ± 3.6 years).[94] The researchers reported that lumbar spine bone mineral density, measured via dual-energy x-ray absorptiometry (DXA), was significantly inversely correlated with 24-hour urinary free cortisol ($p < 0.005$) and morning serum cortisol concentrations ($p < 0.05$) .[94]

Gonzalez et al. assessed the relationship between salivary cortisol concentrations and trabecular bone score (TBS) in 608 female participants who were at least 50 years of age.[95] Trabecular bone score is a measure of bone texture that is positively correlated with microarchitecture and is measured by DXA; higher scores indicate reduced fracture risk.[95,96] Salivary cortisol concentrations were measured at time of awakening, 30 minutes after awakening, at 11:00 AM, and at 8:00 PM. Cortisol was measured at different time points throughout the day to capture the fluctuations in cortisol concentrations that occur as a result of

circadian rhythm. Gonzalez et al. reported that participants in the highest tertile of 8:00 PM salivary cortisol concentrations (mean = 5.7 ± 2.5 nanomoles per liter [nmol/L]) had significantly lower TBS scores than participants with the lowest 8:00 PM salivary cortisol concentrations (mean = 1.7 ± 0.4 nmol/L) ($p = 0.02$).[95]

*2.2.7 Exercise-Induced Cytokine Responses and Effects on Bone*

Researchers' recent conceptualization of skeletal muscle as a secretory organ represents a paradigm shift in the understanding of the endocrine and immunological effects of exercise.[97] Contextualizing these effects helps elucidate the influence of endurance exercise on bone turnover, because the mechanisms involved in bone resorption and formation are driven by many of the same compounds involved in the response to exercise. Exercise also directly affects bone turnover and formation markers.[13]

Interleukin-6 (IL-6) is one of several myokines released from skeletal muscle cells during exercise.[98] Interleukin-6 is pleiotropic, exerting both pro-inflammatory and anti-inflammatory effects, depending on the type of signaling receptor to which IL-6 binds and concentrations of other compounds in the surrounding milieu.[97-99] When bound to its "classical" membrane-bound receptor, IL-6 exhibits anti-inflammatory functions; when bound to a soluble IL-6 receptor (sIL-6R), IL-6 exerts pro-inflammatory functions.[100] The membrane bound receptor is only found on cells of the immune system, myocytes,

hepatocytes, and brain cells.[101] The sIL-6R allows IL-6 to bind cells that do not contain the classical membrane receptor, by instead, facilitating trans-signaling by binding glycoprotein 130, which is expressed by most cells.[100]

Researchers have shown that, when IL-6 is chronically elevated in basal conditions, it is pro-inflammatory.[15,102] As a myokine acutely released by contracting muscles, IL-6 performs several functions that support working muscles. For example, glucose uptake is enhanced by IL-6 inducing the translocation of GLUT-4 receptors to the plasma membrane.[15] Interleukin-6 has also been shown to enhance insulin sensitivity[102] and lipolysis[103] in skeletal muscle, as well as inhibit TNF$\alpha$.[102] Plasma concentrations of IL-6 have been reported to increase following exercise in both untrained participants and highly-trained elite athletes. In a systematic review of exercise protocols in untrained participants, Brown et al. reported that participants' IL-6 concentrations were significantly increased in four of six studies the authors reviewed.[104]

Wallberg et al. measured IL-6 concentrations in elite ultra-endurance athletes completing either a 24-hour laboratory protocol at 60% of maximal oxygen consumption or a six-day, 800 kilometer Adventure Race at roughly 38% of maximal oxygen consumption.[105] For the laboratory protocol, nine male adventure race athletes with three to nine years of experience competing in international elite ultra-endurance events were recruited (mean age = 27 ± 1

years, mean maximal oxygen consumption = 62.5 ± 5.3 mL/kg/min). For the six-day Adventure Race protocol, nine highly-trained male participants (mean age = 30 ± 4 years, mean maximal oxygen consumption = 61.5 ± 2.5 mL/kg/min), three less-trained male participants (mean age = 33 ± 4 years, mean maximal oxygen consumption = 51.8 ± 2.2 mL/kg/min) and six female participants (mean age = 32 ± 7 years, mean maximal oxygen consumption = 55.8 ± 1.8 mL/kg/min) were recruited. All participants had previously competed in the 2006 AdventureRacing World Championship in Hemavan, Sweden.

The study was limited by a small sample size, only male participants were recruited for the laboratory protocol, and three out of 18 participants dropped out of the six-day protocol due to fatigue. Despite this, the researchers reported several significant findings. Interleukin-6 concentrations were significantly increased from a mean baseline value of 0.76 ± 0.48 pg/mL to 10.58 ± 1.04 pg/mL during the first 12 hours of the 24-hour laboratory protocol ($p < 0.001$), after which concentrations plateaued. After 28 hours of rest following the 24-hour laboratory protocol, IL-6 concentrations were still significantly elevated from baseline: mean IL-6 concentration (3.10 ± 2.43 pg/mL; $p < 0.05$).

During the six-day event, three participants dropped from the race due to fatigue. The remaining 15 participants completed the race with a mean finish time of 145.9 ± 10.0 hours. Among the 15 men and women who completed the

six-day Adventure Race, IL-6 concentrations were significantly increased at the end of the race compared to baseline: mean IL-6 concentration was 9.87 ± 5.91 pg/mL at the end of the race and 0.53 ± 0.47 pg/mL at baseline ($p < 0.001$). Interleukin-6 concentrations increased throughout the first 24 hours, but did not significantly differ in the time points between 24 hours and 72 hours into the race. During both protocols, no significant differences in TNF$\alpha$ concentrations were observed.[105] This may be the result of IL-6 attenuating TNF$\alpha$ secretion, or from the highly-trained status of the participants.

The relationship between IL-6 and bone health is not as clear-cut as the evidence demonstrating exercise-induced elevations in IL-6 concentrations. In an *in vitro* experiment, Axmann and colleagues demonstrated that inhibiting IL-6 receptor (IL-6R) with an anti-IL-6R antibody, significantly reduced the number of multinucleated osteoclasts when monocytes were stimulated with receptor activator of nuclear factor kappa-B ligand (RANKL) ($p < 0.01$).[106] However, the researchers also reported that adding IL-6 alone, not only failed to increase osteoclast numbers, but also significantly reduced osteoclast size ($p < 0.05$).[106] Scheller and colleagues reviewed literature on IL-6 and bone metabolism, and explained that osteoclast formation was triggered by IL-6 only when soluble interleukin-6 receptor (sIL-6R) was present in culture. A combination of IL-6 and sIL-6R have been shown in *in vitro* experiments to upregulate RANKL. Scheller

et al. also reviewed research conducted on IL-6 knockout mice, in which researchers reported that the IL-$6^{-/-}$ group was protected from ovariectomy-induced bone loss.[99]

These findings confirm a role for IL-6 and sIL-6R in osteoclast proliferation. However, it is difficult to determine the extent to which IL-6 contributes to reduced bone mineral density among endurance athletes, given the host of potential mechanisms that may contribute to bone resorption. This is an inherent disparity between human versus animal and *in vitro* experiments, particularly when a cross-sectional methodology is employed to explore relationships between variables in humans. Additionally, concentrations of sIL-6R have been shown to vary according to the training volume an athlete completes the previous week before sIL-6R concentrations are measured.[101] These adaptations demonstrate that the relationship between exercise and inflammation is complex; exercise is an acute inflammatory stressor, but the anti-inflammatory compensatory mechanisms built into the body's stress response systems ameliorate acute inflammation.

*2.2.8 Defining Physical Activity Volume Threshold for Reduced Bone Mineral Density Risk*

There is a gap in the literature in terms of quantifying the volume of activity that tips the balance of bone health in the direction of excessive

resorption. Whitfield et al. set out to clarify this quandary by analyzing data from the 2007 to 2010 National Health and Nutrition Examination Survey (NHANES).[34] Self-reported physical activity was used to calculate an estimation of the number of metabolic equivalent (MET) hours per week. The authors defined 7.5 MET hours as meeting minimal physical activity guidelines, 15 to 22.49 MET hours as doubling physical activity guidelines, 22.5 to 29.99 as tripling physical activity guidelines, and above 30 MET hours as quadrupling physical activity guidelines. The 2007 to 2010 NHANES dataset contained 9,486 participants who had femoral bone mineral density (BMD) measured by DXA. Furthermore, there were complete covariate data for age, sex, race/ethnicity, reported calcium and vitamin D supplement use, osteogenic medications and non-steroid anti-inflammatory drug use, oral contraceptive use, and sex hormone therapy. Lumbar DXA was available for 7,787 participants.[34]

Whitfield et al. reported that the relative odds of low lumbar and proximal femur BMD for adults, 20 years of age and older, were not significantly different across physical activity categories. However, the authors reported that women who reported activity between two and four times higher than the physical activity guidelines were significantly less likely to have low lumbar spine BMD compared to women who reported no physical activity (adjusted odds ratio [95% confidence interval] = 0.71 [0.51 to 0.97]). Women who reported

activity between one and two times higher than the physical activity guidelines, and women who reported activity between two and four times higher than the guidelines were significantly less likely to have low proximal femur BMD compared to women who reported no physical activity (adjusted odds ratio [95% confidence interval] = 0.77 [0.60 to 0.99], 0.70 [0.54 to 0.91], respectively). There was no significant reduction in risk for low lumbar spine BMD among men who reported activity between two and four times the physical activity guidelines compared to men who reported no activity (adjusted odds ratio [95% confidence interval] = 0.91 [0.70 to 1.19]). However, men who reported activity between four and six times the physical activity guidelines had significantly lower relative odds of low lumbar spine BMD compared to men who reported no activity (adjusted odds ratio [95% confidence interval] = 0.51 [0.32 to 0.81]). Similar odds ratios were reported for men who reported between six and eight times the physical activity guidelines, and men who reported greater than eight times the physical activity guidelines, compared to men who reported no activity (adjusted odds ratios = 0.57, 0.52, respectively).

Multivariable-adjusted mean lumbar and proximal femur BMD were calculated for men and for women that adjusted for age, race/ethnicity, body mass index, osteogenic medication use, alcohol consumption, and smoking status. Men reporting physical activity between four and six times higher than

the guidelines had significantly higher lumbar spine BMD (multivariable-adjusted mean = 1.088 g/cm$^2$) compared to those not meeting the physical activity guidelines (multivariable-adjusted mean = 1.042 g/cm$^2$, $p$ = 0.001). Women reporting two to four times higher than the physical activity guidelines had higher proximal femur BMD (multivariable-adjusted mean = 0.928 g/cm$^2$) than those not meeting the physical activity guidelines (multivariable-adjusted mean = 0.904 g/cm$^2$, $p$ = 0.018).[34]

The authors report that the range of activity volumes reported by NHANES participants is lower than the physical activity volumes of athletes in training, and thus may explain the discrepancy between their findings and the findings of others.[17,18,107] For example, Whitfield et al. calculated the number of MET hours per week in competitive basketball players as 186 MET hours per week, which is approximately 25 times the physical activity guidelines.[34] More research is needed to help establish the physical activity volume threshold associated with reduced bone mineral density.

**2.3 Relationships between Dietary Intake and Bone Health**

Calcium intake, protein intake, and vitamin D derived from both the diet and the sun are three nutritional factors that influence bone health. It is pertinent to summarize the roles of these three nutrients on bone health, because they may influence the relationship between soluble and total fiber and BMD in endurance

athletes. Calcium, vitamin D, and protein are potential covariates in the relationship between fiber and BMD because they directly augment BMD. Additionally, each of these nutrients may mediate the relationship between fiber and BMD at the level of the gut.

The role of calcium in enhancing bone health is widely known.[7,108] The Dietary Reference Intakes (DRI) for calcium intake among children and adolescents were created based on the quantity of calcium intake demonstrated by researchers to contribute to bone accretion and positive calcium balance.[108] The DRI for adults was created based on calcium intakes demonstrated by researchers to maintain bone heath and calcium balance. The Recommended Dietary Allowance (RDA) for calcium is 1,000 mg per day for men and women, 19 through 50 years of age. Among individuals 51 through 70 years of age, the RDA is 1,000 mg per day for men and 1,300 mg per day for women. Weaver et al.[7] identified nine randomized, controlled trials in which the effects of calcium supplements on BMD were assessed. Researchers reported increased BMD among participants administered calcium supplements in eight of the nine trials.[7]

Vitamin D is another micronutrient that plays a pivotal role in bone health. Vitamin D may be produced endogenously through a photolytic process in the skin that converts 7-dehydrocholesterol into calcidiol when the skin is exposed to ultraviolet B (UVB) rays from the sun.[109] Calcidiol is converted to the

active form (calcitriol) by enzymatic conversions in the liver and kidney.[109] Vitamin D may be consumed from dietary sources such as fatty fish, egg yolks, vitamin D fortified foods, and supplements.[110] The RDA for vitamin D was calculated based on the median intake value of vitamin D required to maintain serum calcidiol concentrations between 30 and 50 nanomoles per liter (nmol/L). The 30 to 50 nmol/L range has been shown by researchers to enhance calcium absorption and protect against decreased BMC.[108] The RDA for vitamin D is 600 International Units (IU) for men and women between one and 70 years of age.[108] The integral role of vitamin D in maintaining bone health renders the consideration of this nutrient essential to studying variables that may affect bone. Vitamin D may also mediate some of the pro-inflammatory effects of exercise. Willis and colleagues reported a significant inverse relationship between serum calcidiol concentrations and serum TNF$\alpha$ concentrations ($p < 0.001$) in a sample of nine male (mean age 27.4 ± 9.4 years) and ten female (mean age 29.1 ± 7.5 years) endurance trained runners, who ran between 30 and 85 kilometers per week.[111]

Heaney and Layman reviewed studies in which researchers explored the relationship between protein intake and bone health.[112] While the authors acknowledge that different types of protein have been reported to exert varied effects on bone mass, they concluded that overall higher protein diets were

associated with increased bone mass and reduced fracture risk when calcium intake was adequate.[112] Similarly, Bonjour reported in a review of the role of nutrition on bone health, that protein intake was positively related to BMC and BMD.[113] Weaver and colleagues identified four prospective studies published after the year 2000 in which researchers demonstrated positive findings for the relationship between protein intake and BMC.[7] The authors identified one randomized controlled trial in which a 42-gram protein supplement was not reported to enhance bone mineral content, and assigned an overall grade of evidence of C: limited to protein as a lifestyle factor for augmenting bone health.[7] Clarke and colleagues employed 16S rRNA sequencing of fecal samples to assess the population composition of the gut microbiome in a sample of 40 male elite professional rugby players (mean age = 29 ± 4 years) and 46 healthy male controls (mean age = 29 ± 6 years).[114] The researchers also assessed dietary intake from food frequency questionnaires, and reported that grams per day of protein intake correlated positively with the number of observed microbial species ($p$ = 0.007). While these findings are mixed, the role of protein as contributing 50% of bone volume[112] render this nutrient an important consideration for assessing bone health.

## 2.4 Conclusion

This literature review has presented research demonstrating the body's acute inflammatory response to endurance training, the fermentation of fiber by the microbiome, and the role of short-chain fatty acids in attenuating inflammation and enhancing mineral absorption and bone mineral density. Inflammatory responses, though attenuated by anti-inflammatory feedback regulation, pose potentially harmful consequences for gut and bone health. It is important to remember that the body's regulation of bone health is highly nuanced and complex. Although the immunological and inflammatory mechanisms described here do contribute to bone resorption, overall physical activity is a well-documented contributor to bone mineral density.[7] Mechanical loading contributes to bone modeling, rendering physical activity an important contributor to bone mass and density during periods of peak bone accrual.[7] The highest level of evidence (A) was assigned to the effect of physical activity and exercise on bone mass and density by the National Osteoporosis Foundation.[7]

However, in their systematic review, Weaver et al. note that more research is needed to determine the dimensions, dose, and timing of exercise that is needed to confer a maximum benefit to bone.[7] The mechanism of mechanical loading may not apply to highly trained endurance athletes. Increased mechanical loading through impact and muscle contraction activates a signal

cascade from mechanosensitive osteocytes to activate osteoblasts and osteoclasts, a mechanism requiring that the strain to bone must be greater than the habitual level of strain.[7] Endurance athletes who perform the same repetitious motions (such as running or cycling) may not be stimulating osteocytes to initiate the signal cascade that activates osteoblasts and osteoclasts because they have trained to the maximum mechanical load of their sport and body weight. The lack of mechanical loading may contribute to the reduced lumbar spine bone mineral density observed in elite endurance athletes.

Regardless of the reasons for reduced bone mineral density among endurance athletes, research is needed to ameliorate this problem. Exercise is among the most important behaviors an individual can perform to reduce his or her risk for chronic disease and improve overall health. Researchers should be engaged in the task of defining nutritional and training protocols to ensure that the many benefits of exercise continue to outweigh the costs. Exploring fiber intake among endurance athletes as a potential contributor for attenuating reduced bone mineral density is part of that project.

# CHAPTER 3: METHODOLOGY

## 3.1 Institutional Review Board Approval

The data for the present study were derived from the on-going cross-sectional study: "A Comparison of Fitness Variables in Collegiate Athletes, Reserve Officers' Training Corps (ROTC) Cadets and Midshipmen, and Masters Athletes", referred to in the Specific Aims in Chapter 1, as "The Drexel Fitness Study". The Drexel University Institutional Review Board (IRB) has approved the study as well as all recruitment methods and materials. The purpose of the study was to compare the physical fitness profile of Collegiate athletes, Reserve Officers' Training Corps (ROTC) Cadets and Midshipmen, and Masters athletes at Drexel University and in the surrounding areas. The present study did not utilize all of the data points that have been collected in the full IRB-approved study, thus the methodology described presently encompasses only the measurements that were relevant to the present study.

## 3.2 Exclusion and Inclusion Criteria

The exclusion criteria for the IRB approved study protocol are as follows: smokers, sedentary individuals (defined as persons who exercise less than two days a week), adults unable to consent, individuals under 18 years of age, pregnant women, and prisoners. In addition to these exclusion criteria, the present study applied the following exclusion criteria: individuals prescribed

immuno-suppressing medications (such as glucocorticoids), individuals prescribed medications shown to affect calcium absorption (such as proton pump inhibitors), and individuals with metal implants, such as plates, rods, or screws, because these would confound bone mineral density measures.

The inclusion criteria for the IRB-approved study protocol are as follows: at least 18 years of age and older, and exercising at least two days a week. In addition to these inclusion criteria, the present study applied the following inclusion criteria: individuals who self-reported the sport in which they participated as an endurance sport. Fink and Mikesky define endurance athletes as individuals engaged in continuous activity that involves large muscle groups and lasts between 30 minutes and 4 hours.[115] Ultra-endurance athletes engage in continuous activity that exceeds four hours.[115] Both endurance athletes and ultra-endurance athletes were included for analyses. Ultra-endurance athletes were included for analyses as endurance athletes based on their self-reported sport; thus, no distinction was made during data collection between endurance and ultra-endurance athletes. Upon inclusion into this study, participants were assigned a participant identification that coded their self-reported sport. Participants who defined themselves as a: runner, triathlete, cyclist, rower, soccer player, field hockey player, ice hockey player or swimmer were included in the present study.

### 3.3 Recruitment

Athletes were recruited through recruitment flyers **(Appendix A)** posted around Drexel University as well as by word-of-mouth. Based on interest, potential participants contacted the project manager via the study email. Each potential participant completed an online survey via Drexel's Qualtrics survey system **(Appendix B)**. After completion of the online survey, the researchers reviewed the survey to ensure that the participant qualified for the study protocol. If a participant did not qualify, he or she was notified via email. If a participant qualified, he or she was contacted via email and/or phone to schedule the first appointment. Reminder emails were sent the day before the first and second appointments. Each athlete participated in the study for two testing appointments, which were usually separated by approximately one week. Both sessions were held at the Nutrition Sciences Metabolic Laboratory (1601 Cherry Street, Room 325A and Room 203).

### 3.4 Laboratory Measurements

#### *3.4.1 Informed Consent and Relevant Questions*

The informed consent document **(Appendix C)** was provided to participants during the first appointment. The participant read the document at his or her own pace, after which one of the researchers discussed the document with the participant and verbally described how the tests were conducted and

the information provided by each test. The researcher answered any questions the participant had regarding the study protocol, and if the participant chose, he/she initialed each page of the consent form and signed the document, and the researcher signed it as a witness. Participants who chose not to sign the consent form were escorted out of the building by one of the researchers.

After signing the informed consent, participants were asked to state any medications they were taking, list any medical conditions/diagnoses, report any specialized diets to which they adhered, state the number of hours of sleep they obtained the night prior, and state the quantity of water they consumed the morning of their appointment. Female participants were asked to report the first date of their last menstrual cycle. Participants were asked if they adhered to the pre-study protocol, which consisted of abstaining from food and caffeine for 12 hours before their appointment time, as well as abstaining from alcohol and exercise for 24 hours before their appointment time. The participants' responses to these questions were recorded on the data collection sheets **(Appendix D)**. If the participant did not adhere to the respective protocol, testing could not be conducted on that particular day.

**3.4.2 Pregnancy Test (Female Participants)**

A self-administered pregnancy test was given to each female participant while the researcher waited outside of the restroom. The researcher confirmed

negative results of the pregnancy test to continue participant inclusion in the study. If the results of the test were positive for pregnancy, the participant was excluded from the study and escorted out of the building by one of the researchers. The pregnancy test was required as a precaution, because there is a small radiation exposure from the dual-energy X-ray absorptiometer.

### 3.4.3 Anthropometrics

A member of the research team collected anthropometric data, while a second member of the research team recorded the results of each measurement on the data collection sheet. Participants' height and body weight were measured twice. Height was measured to the nearest 0.5 centimeters (cm) with a sliding vertical scale stadiometer (Seca, Chino, CA). Weight was measured to the nearest 0.25 pounds (lbs) with a calibrated balance beam scale (Seca, Chino, CA). Waist circumference was measured in triplicate. Waist circumference was measured 1.0 inch above the umbilicus to the nearest 0.1 cm with a soft measuring tape. The average for each measurement was calculated and recorded on the data collection sheet. Statistical analyses were performed on the average value for each anthropometric variable.

### 3.4.4 Dual-Energy X-Ray Absorptiometry (DXA)

Dual-energy X-ray absorptiometry was used to assess bone mineral density (BMD). The DXA scanning arm emits two types of x-ray energy waves.

Software calculated the absorption of each energy wave to differentiate between bone and soft tissue. Bone mineral density was calculated by dividing bone mineral content (BMC) (grams) by bone area ($cm^2$). The DXA instrument (Lunar iDXA, enCORE version 15.0, GE Healthcare, Madison, WI) was calibrated before each measurement with a quality control phantom box to ensure reliability. The use of DXA posed minimal risk to the participant; participants were exposed to fewer radiation rays than would be incurred during a cross-country plane flight.

Participants did not wear any metal for the scans because metal would be mistakenly interpreted as bone tissue by the DXA software. Any jewelry, zippers, grommets, clips, etc., would confound the results of BMD measures. Therefore, participants were instructed to remove shoes and all metal prior to the scans. If a participant had metal on his or her clothing (such as a zipper or grommet), a hospital-type gown was provided to the participant by a member of the research team. A retractable curtain was extended in the DXA lab to ensure the privacy of each participant if he or she was required to wear the hospital-type gown. Once all metal was removed, participants were instructed to lie on the DXA table in alignment with the measuring guide printed on the table. A total of four scans were taken: one total body scan; one lumbar spine scan (L1 to L4); and two dual femoral neck scans, one of the left and right dual femoral neck.

For the total body scan, the DXA scanner arm moved the length of the participant's body from head to toe. For the lumbar spine scan, the researcher positioned the DXA scanner arm approximately two inches below the umbilicus and the arm moved the length of the participant's torso from below the iliac crest to L1. For the dual femoral neck scans, the researcher positioned the DXA scanner arm on the participant's upper thigh and the arm moved from the top of the femur to just below the iliac crest (for the right and left femur). At no point in any of these measurements did the DXA scanner arm make direct contact with the participant.

The total body scan provided total body BMD (grams/$cm^2$), BMC (grams), and information on body composition. This included percent body fat, total mass, tissue mass, lean body mass, and fat-free mass. The total body scan provided a breakdown of each of these variables according to total and left and right body region (arms, legs, trunk, and total body). Body composition was plotted as a percentile according to a population distribution obtained from combined National Health and Nutrition Examination Survey (NHANES) and Lunar data. A color mapping image was provided showing high fat and lean regions of the body. The GE Medical Systems Lunar software (Lunar iDXA, enCORE version 15.0, GE Healthcare, Madison, WI) also calculated a young adult T-score and age-matched Z-score from total body BMD. Total body BMD

was plotted on a population distribution obtained from combined NHANES and Lunar reference populations[116] Site-specific BMD was provided for the head, arms, legs, trunk, ribs, spine, and pelvis. Left side of the body and right side of the body BMD were provided for arms, legs, trunk, and total body.

The lumbar spine scan provided individual BMD (grams/cm$^2$), BMC (grams), area (cm$^2$), width (cm), and height (cm) for L1, L2, L3, L4, and L1-= to L2, L1 to L3, L1 to L4, L2 to L3, L2 to L4, and L3 to L4. The GE Medical Systems Lunar software calculated the young-adult T-score and the age-matched Z-score for each of these regions, and plotted L1 to L4 BMD on a population distribution obtained from combined NHANES and Lunar data. The dual femoral neck scans provided mean BMD (grams/cm$^2$), BMC (grams), area (cm$^2$), the young-adult T-score, and the age-matched Z-score for both the left femoral neck and the right femoral neck. Mean BMD for both the left and right femoral neck were plotted on a population distribution obtained from combined NHANES and Lunar data.

After the four scans were taken, a member of the research team recorded the data onto the data collection sheets. The researcher showed all scans to the participant and explained that the results are used for research rather than diagnostic purposes. The participant was informed that he or she may take copies of the scans to his or her physician, if desired. The researcher answered any questions the participant had regarding the scan results, under the caveat

that such explanations did not qualify as medical advice. The participant was informed that his or her scans would be sent via email as a .pdf file, and the researcher sent a .pdf of the DXA scans to the participant before his or her second session.

### 3.4.5 Actical™ Accelerometer

The participant received an Actical™ accelerometer during the first study session. A member of the research team instructed the participant on the use of the device. This model was a triaxial accelerometer, and measured movement in the vertical, horizontal, and mediolateral planes. The accelerometer was worn on the participant's non-dominant wrist, right hip, or right ankle for seven days. Placement depended on the nature of the sport in which he or she participated, because specific placement may have created hindrances to performance in certain sports. During the seven days while they were wearing the accelerometer, participants were also asked to keep an Activity Log of their exercise to provide the researchers detailed information regarding the type of activity performed to help classify the data collected by the accelerometers **(Appendix E)**. They were instructed to press the "event marker" on the accelerometer to label intentional physical activity in the accelerometer data output. The participants returned the accelerometer and Activity Log to the Metabolic Lab at their second scheduled session.

If for whatever reason the Actical™ accelerometers could not be used at that time (i.e., they were not working, out for service, or there were none available), data collection continued without them. The participant would not be given an accelerometer after the first session. If amenable to the participant, he or she would be given an accelerometer to wear for one week at a time other than between their first and second sessions. This allowed the researchers to still collect the accelerometry data, even if it did not take place between the initially scheduled appointments.

The Actical™ accelerometers provided the total number of minutes spent in sedentary, light, moderate, and vigorous activity. The average kilocalories per minute expended in each of these activity states were also provided. From these data, activity level was assessed. Participants in the upper quartile of total minutes spent in combined moderate and vigorous physical activity were compared to participants in the lower quartile of combined total minutes spent in moderate and vigorous physical activity to determine if activity level influenced the relationship between soluble fiber intake and lumbar spine bone mineral density. Missing data were expected due to the limited number of accelerometers available to distribute to participants. Therefore, these analyses were conducted in the subset of participants for whom accelerometer data were collected.

### 3.4.6 Food Frequency Questionnaire (FFQ)

During the second session, participants were asked to complete a self-administered 2005 Block Food Frequency Questionnaire (FFQ) (NutritionQuest, Berkeley, California). The scan-tron format FFQ took approximately 30 to 40 minutes to complete. A portion size guide was provided with the questionnaire to help the participants choose the appropriate portion size that best reflected their intake. A member of the research team was available to answer any questions participants may have had regarding the FFQ throughout their completion of the FFQ. The FFQ evaluated participants' dietary patterns over the past year. The full-length questionnaire contained approximately 110 food items as well as supplements. The food list for the questionnaire was derived from NHANES 1999 to 2002 dietary recall data. The nutrient database was developed from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies (FNDDS), version 1.0.[117]

A member of the research team collected the completed questionnaires and stored them in the locked file cabinet in the Metabolic Lab (1601 Cherry Street, Room 325A). The FFQs were sent out for analyses by a third-party group (NutritionQuest, Berkeley, California). Before sending the FFQs to be analyzed, the FFQs were sorted by identification number and all identification numbers were entered into a Microsoft® Excel (Microsoft®, Redmond, WA, 2010)

spreadsheet. When the FFQs were received from NutritionQuest, the list of returned FFQ identification numbers were cross-referenced with the sent list to ensure that no data were lost or misplaced. Results were returned from NutritionQuest as a .txt file on a CD-ROM that were imported into Excel. The original FFQs were also returned and archived in a locked file room. The returned results provided a detailed diet analysis for each participant that included daily average energy, macronutrient, micronutrient, and food group serving intakes. From these data, dietary intake data were obtained (macronutrients; total fiber; soluble fiber; fiber from beans, fruits, vegetables, and grains; average daily servings of fruits, vegetables, dairy, and grains; calcium; and vitamin D).

**3.5 Data Management**

All data were collected and documented on hand-written data collection sheets before electronic input. Data collection sheets only used participant identification numbers. All forms were stored in a locked cabinet in the locked Metabolic Laboratory, Room 325A of 3 Parkway (1601 Cherry Street). Only members of the research team had access to the data. Data were transferred from paper collection sheets to Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corp., Armonk, NY, 2016). The SPSS master spreadsheets were exported to Microsoft® Excel (Microsoft®, Redmond, WA, 2010). Results of the

third-party analyses were entered into Microsoft® Excel and SPSS databases. All electronic files were stored on an encrypted computer in the Metabolic Laboratory.

**3.6 Statistical Analyses**

*3.6.1 Overall Analyses*

Data were analyzed using standard SPSS v. 24 software with the alpha set *a priori* at 0.05. Descriptive statistics were performed to determine means, medians, ranges, and standard deviations of participants' age, anthropometrics, lumbar spine bone mineral density, fat free mass, and dietary intake variables. The data were assessed for normality using the Kolmogorov-Smirnov (K-S) test to determine the appropriateness of conducting parametric tests. A significant ($p < 0.05$) finding indicated that the assumption of normality was violated, and non-parametric analyses were used to analyze those variables found to have a non-normal distribution. One caveat of the K-S test is an increased likelihood of a significant result as sample size increases. As a second measure to assess normality, skewness was divided by standard error of skewness for those variables with a significant K-S statistic. If the quotient was within the range of -1.96 and +1.96, parametric tests were employed.

Nonparametric median tests were conducted to determine sex differences between age, body weight, energy intake, total fiber intake, soluble fiber intake,

total calcium intake, total vitamin D intake, protein intake as percent of total energy intake, and time spent in moderate and vigorous physical activity. Researchers have demonstrated that calcium[108], vitamin D[108-110], and protein[7,112] influence bone health. Therefore, these nutrients were considered as potential covariates.

Independent samples t-tests were conducted to determine sex differences between height, fat-free mass, and anterior-posterior lumbar spine bone mineral density. These descriptive analyses were repeated in the subset of participants for whom accelerometer data were available.

The Food and Nutrition Board of the National Academy of Sciences has recommended an Adequate Intake (AI) guideline for total fiber consumption for men and women.[29] This guideline is 14 grams of fiber for every 1,000 kilocalories consumed.[29] To assess whether participants met this guideline, a variable was computed to determine each participant's recommended total fiber intake. Average daily energy intake was divided by 1,000 and this quotient was multiplied by 14. Individuals with a total fiber consumption greater than the calculated value were categorized as meeting AI recommendations. Individuals with a total fiber consumption less than the calculated value were categorized as not meeting AI recommendations. Using the 14 grams per 1,000 kilocalorie intake recommendation has two advantages. First, it was independent of age

and sex. Second, this method circumvented the underreporting limitation inherent to food frequency questionnaires by determining AI fiber intake from within the dataset.

### 3.6.2 Analyses for Specific Aims

#### *3.6.2.1 Specific Aim 1*

To determine the relationship between soluble fiber intake and anterior-posterior lumbar spine lumbar bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

To determine the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes, Spearman's rank correlation coefficient was calculated. Separate Spearman's rank correlation coefficients were calculated between anterior-posterior lumbar spine BMD and dietary intake variables to assess whether any of these variables significantly influenced anterior-posterior lumbar spine BMD. A zero-order Pearson Product Moment Correlation was calculated between anterior-posterior lumbar spine BMD and fat-free mass because both variables presented normal distributions. A

partial Spearman's rank correlation coefficient was calculated between anterior-posterior lumbar spine BMD and soluble fiber intake that controlled for the variance in lumbar spine BMD contributed by fat-free mass, dietary vitamin D intake, and body mass index.

*3.6.2.2 Specific Aim 2*

To determine the combined effects of calcium and soluble fiber intakes on anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

The combined effects of calcium and soluble fiber intake on anterior-posterior lumbar spine bone mineral density was unable to be assessed because the dietary intake data were not normally distributed. This violated the assumption of normality that is needed to proceed with regression analyses. Currently, SPSS does not offer nonparametric regression analyses. Rather than calculate a regression equation, the sample was divided into two groups: participants who met Recommended Dietary Allowances (RDA) for calcium intake, and participants who did not meet RDA. A Spearman's rank correlation

coefficient was calculated for each group. A partial Spearman's rank correlation coefficient was also calculated for each group to control for the variance in anterior-posterior lumbar spine BMD contributed by fat free mass, dietary vitamin D intake, and body mass index. Regression slopes were calculated for the relationship between soluble fiber intake and anterior-posterior lumbar BMD for both the group of participants who met the calcium RDA and the group of participants who did not meet the calcium RDA. A *z*-test was performed to assess whether the differences between the two slopes were significant. A significant finding was defined as $z = \pm 1.96$.

*3.6.2.3 Specific Aim 3*

To determine the relationship between total fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

To determine the relationship between total fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes, a Spearman's rank correlation coefficient was calculated. A partial Spearman's rank

correlation coefficient was also calculated to control for the variance in anterior-posterior lumbar BMD contributed by fat-free mass, dietary vitamin D intake, and body mass index.

*3.6.2.4 Specific Aim 4*

To determine if sex influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined. To determine if sex influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes, individual Spearman's rank correlation coefficients were calculated in female participants and male participants and the results of each correlation were compared. A partial Spearman's rank correlation coefficient was also calculated for each quartile to control for the variance in anterior-posterior lumbar BMD contributed by fat-free mass, body mass index, and dietary vitamin D intake. Chi-square tests were applied to the relationship between men and women meeting nutrient needs for fiber, between men and women meeting nutrient needs for calcium, between

men and women meeting nutrient needs for vitamin D, and between men and women meeting both calcium and fiber needs to determine whether sex influenced the probability of meeting nutrient needs. Regression slopes were calculated for the relationship between soluble fiber intake and anterior-posterior lumbar BMD for both male participants and female participants. A *z*-test was performed to assess whether the differences between the two slopes were significant. A significant finding was defined as $z = \pm 1.96$.*3.6.2.5*

*Specific Aim 5*

To determine if time spent in combined moderate and vigorous physical activity influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined.

To determine whether time spent in combined moderate and vigorous physical activity affected the relationship between soluble fiber intake and anterior-posterior lumbar spine BMD, the median value of minutes spent in total moderate and vigorous physical activity was used to divide the sample into: 1)

the lower quartile of time spent in total moderate and vigorous physical activity and 2) the upper quartile of time spent in total moderate and vigorous physical activity.

Individual Spearman's rank correlation coefficients were calculated on first the lower quartile of time spent in moderate to vigorous physical activity and then the upper quartile of time spent in moderate to vigorous physical activity. A partial Spearman's rank correlation coefficient was also calculated for each quartile to control for the variance in lumbar BMD contributed by fat-free mass, dietary vitamin D intake, and body mass index. Regression slopes were calculated for the relationship between soluble fiber intake and anterior-posterior lumbar BMD for both the lower quartile group and the upper quartile group. A *z*-test was performed to assess whether the differences between the two slopes were significant. A significant finding was defined as $z = \pm 1.96$.

## CHAPTER 4: JOURNAL MANUSCRIPT

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## INTRODUCTION

Dietary recommendations to athletes are tailored to enhance performance and accelerate recovery. Recommendations focused on these parameters are rarely made in consideration of gut or bone health. It is the position of both the American College of Sports Medicine (ACSM) and the Academy of Nutrition and Dietetics (AND) that athletes should consume low-fiber foods prior to physical activity to prevent gastrointestinal complications and ensure rapid delivery of carbohydrate during activity.[1] The current body of nutrition recommendations for athletes does not emphasize fiber consumption during other times, outside of blanket recommendations to follow a healthy eating pattern.[2] These recommendations, paired with a lack of emphasis on fiber-rich foods during times when athletes are not competing, may negatively influence athletes' fiber consumption.

Fiber may play important roles in enhancing mineral absorption, attenuating intestinal permeability, reducing lipopolysaccharide (LPS) translocation, and modulating the immune system via fermentation by the microbiota into bioactive short-chain fatty acids.[3-5] These mechanisms have important implications for bone health, and diets rich in fiber may contribute to increased bone mineral density.[6] A 2016 systematic review and position statement of the National Osteoporosis Foundation explained that increased

consumption of fermentable fibers has been positively associated with calcium absorption.[7] However, only one study reviewed by the authors was of long enough duration to measure changes in bone mineral content (BMC) and bone mineral density (BMD). More studies are needed to understand the effect of fiber on bone health.

The National Osteoporosis Foundation has assigned a strong level of evidence (A) to the effect of physical activity and exercise on bone mass and BMD.[7] However, endurance athletes may not be engaged in the types of activities that maximize the dynamic, high magnitude, short duration, or high impact movements that have been reported to be most osteogenic. Endurance athletes engaged in activities such as running, cycling, swimming, and other not weight-bearing sports tend to have lower BMD than athletes engaged in weight-bearing sports and individuals who are not physically active.[8] Researchers consistently report that, although runners have higher BMD at primary impact sites (such as the calcaneus and tibia), overall BMD is lower than athletes competing in sprinting, gymnastics, cycling, and ball sports.[9,10,11] Reduced BMD across these samples of endurance athletes indicates a need for researchers to elucidate factors driving the discrepancy between the known benefits of exercise and the cost of prolonged endurance exercise to bone health.

The potential for fermentable fiber to promote bone health, and perhaps attenuate some of the risks associated with prolonged endurance training, is intriguing. Researchers have demonstrated a role for fermentable fiber in augmenting bone health based on findings from animal models, *in vitro* experiments, and supplementation trials in adolescents.[12-15] Previous research on fiber intake and BMD in female athletes has shown that increased fiber consumption correlates negatively with BMD.[16,17] However, these studies were conducted on participants with oligomenorrhea and amenorrhea; participants with eumenorrhea either comprised a small percent of the samples or were excluded entirely. Furthermore, little has been studied on the effects of fiber intake on BMD in healthy female athletes. The conclusions drawn from these studies should be reevaluated in a healthy population.

We measured anterior-posterior (AP) lumbar spine BMD from L2 to L4 and dietary intake variables in a sample of healthy male and female endurance athletes to assess the relationship between soluble and total fiber intakes and bone mineral density. The specific aims and hypotheses of this study are: 1) To determine the relationship between soluble fiber intake and anterior-posterior lumbar spine lumbar bone mineral density in athletes, 18 years of age and older, who self-reported endurance sports as their primary physical activity and participated in the cross-sectional Drexel Fitness Study. The Drexel Fitness

Study is a long-term, cross-sectional study, where athletes' body weight, height, waist circumference, resting metabolic rate, maximal oxygen consumption, bone mineral density and dietary intake are examined. These athletes represent a novel population for investigating this relationship. The lumbar spine was chosen to reduce the potential confounding effects of mechanical loading that might enhance dual femoral neck BMD among runners, since not all of the endurance athletes in the sample reported running as their primary activity. It was hypothesized that soluble fiber intake would be positively correlated with bone mineral density in endurance athletes.

2) To determine the combined effects of calcium and soluble fiber intakes on anterior-posterior lumbar spine bone mineral density in the aforementioned endurance athletes. Since one of the proposed mechanisms for fiber enhancing BMD is enhanced calcium absorption, it was hypothesized that higher combined calcium and soluble fiber intake will have a greater positive influence on anterior-posterior lumbar spine bone mineral density.

3) To determine the relationship between total fiber intake and anterior-posterior lumbar spine bone mineral density in the aforementioned endurance athletes. It is hypothesized that individuals meeting Adequate Intake guidelines for fiber will have higher anterior-posterior lumbar spine bone mineral density compared to individuals who do not meet Adequate Intake guidelines. There are no

Adequate Intake guidelines for soluble fiber, and soluble fiber is a component of total fiber. Thus, this aim explores whether meeting Adequate Intake recommendations for fiber contribute in a meaningful way to bone health.

4) Due to the sex-specific characteristics of bone metabolism across the lifespan, we also aimed to evaluate sex-specific differences in the relationship between soluble fiber and bone mineral density in the aforementioned group of endurance athletes. It was hypothesized that the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in endurance athletes will be stronger among male participants compared to female participants.

5) To determine if activity level influences the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density in the aforementioned group of endurance athletes. It is hypothesized that the relationship between soluble fiber intake and anterior-posterior lumbar spine bone mineral density will be stronger among participants spending more time in moderate and intense physical activity.

It has been well-established that calcium[18], vitamin D[18-20], and protein[7,21] influence bone health. Therefore, these nutrients were considered as potential covariates.

## METHODS

Data for the present study were derived from an on-going, cross-sectional study ("Drexel Fitness Study") in the Department of Nutrition Sciences at Drexel University in Philadelphia, Pennsylvania. Drexel University Institutional Review Board (IRB) approved the study protocol and materials prior to data collection. All participants provided written informed consent.

### Inclusion and Exclusion Criteria for the Study Protocol

Potential participants completed an online survey to assess his or her eligibility for inclusion in the study. If qualified, the participant was contacted via phone or email to schedule the first testing session. Exclusion criteria were: smokers, sedentary individuals (defined as persons who exercise less than two days a week), adults unable to consent, individuals under 18 years of age, pregnant women, and prisoners. The inclusion criteria for the IRB-approved study protocol were: individuals who are at least 18 years of age and exercise at least two days a week.

The following exclusion criteria were applied for data analyses: individuals prescribed immuno-suppressing medications, individuals prescribed medications shown to affect calcium absorption, individuals with metal implants that would confound bone mineral density measures, and individuals who self-report their primary activity as one not defined as an endurance sport. Fink and

Mikesky define endurance athletes as individuals engaged in continuous activity that involves large muscle groups and lasts a minimum of 30 minutes.[22] Upon inclusion into this study, participants were assigned a participant identification that coded their self-reported sport. Participants who defined themselves as one or more of the following: runner, triathlete, cyclist, rower, soccer player, field hockey player, ice hockey player or swimmer were included in the present study.

**Data Collection**

Participants came to the Drexel University Nutrition Sciences Metabolic Laboratory (1601 Cherry Street, Rooms 325A and 203) for a total of two sessions. Although more data were collected for the larger, on-going study, only data collection pertinent to this study will be described. During the first session, anthropometric measurements were taken and dual-energy X-ray absorptiometry (DXA) scans were conducted. A self-administered pregnancy test was administered to each female participant due to the DXA scan. During the second session, dietary intake was assessed by a self-administered 2005 Block Food Frequency Questionnaire (FFQ).

**Anthropometrics**

Participants' height and body weight were measured in duplicate. Height was measured to the nearest 0.5 centimeters (cm) with a sliding vertical scale stadiometer (Seca, Chino, CA). Weight was measured to the nearest 0.25 pounds

(lbs) with a calibrated balance beam scale (Seca, Chino, CA). Waist circumference was measured in triplicate. Waist circumference was measured 1.0 inch above the umbilicus to the nearest 0.1 cm with a soft measuring tape. Mean values were obtained for each measure and used for statistical analyses.

**Bone Mineral Density and Fat-Free Mass**

Dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Madison, WI) was used to assess body composition and bone health. Bone mineral content (BMC) (grams) and areal bone mineral density (aBMD) (grams/$cm^2$) of the total body, left and right femoral necks, and anterior-posterior lumbar spine were determined. Fat-free mass (FFM) (kilograms [kg]), lean body mass (LBM) (kg), and percent body fat were also assessed.

**Physical Activity**

The participant received an Actical™ accelerometer at the end of the first study session. This model was a triaxial accelerometer, and measured movement in the vertical, horizontal, and mediolateral planes. Participants were instructed to keep an Activity Log of their exercise and to press the "event marker" on the accelerometer to label intentional physical activity in the accelerometer data output. The accelerometers provided the total number of minutes spent in sedentary, light, moderate, and vigorous activity. The average kilocalories per minute expended in each of these activity states were also provided. Missing

data were expected due to the limited number of accelerometers available to distribute to participants. Therefore, analyses were conducted in the subset of participants for whom accelerometer data were collected. Participants' physical activity levels were compared to the American College of Sports Medicine's physical activity guidelines, defined as 150 minutes per week of moderate intensity cardiorespiratory training,[23] to determine whether participants met these physical activity guidelines.

**Dietary Intake**

During the second session, participants were asked to complete a self-administered 2005 Block Food Frequency Questionnaire (FFQ) (NutritionQuest, Berkeley, California). The scan-tron format FFQ took approximately 30 to 40 minutes to complete. A portion size guide was provided with the questionnaire to help the participants choose the appropriate portion size that best reflected their dietary intake. Researchers were present to answer any questions and to assure that the FFQ was completed properly. The FFQ evaluated participants' dietary patterns over the past year. The full-length questionnaire contained approximately 110 food items as well as supplements. The food list for the questionnaire was derived from National Health and Nutrition Examination Survey (NHANES) 1999 to 2002 dietary recall data. The nutrient database was developed from the United States Department of Agriculture (USDA) Food and

Nutrient Database for Dietary Studies (FNDDS), version 1.0.[24] The FFQs were sent out for analyses by a third-party group (NutritionQuest, Berkeley, California). The returned results provided a detailed diet analysis for each participant that included daily average energy, macronutrient, micronutrient, and food group serving intakes. From these data, dietary intake data were obtained (macronutrients; total fiber; soluble fiber; fiber from beans, fruits, vegetables, and grains; average daily servings of fruits, vegetables, dairy, and grains; calcium; and vitamin D).

The Food and Nutrition Board of the National Academy of Sciences provides a total fiber Adequate Intake (AI) recommendation of 14 grams of fiber for every 1,000 kilocalories consumed for both men and women.[25] To assess whether participants met this guideline, a variable was computed to determine each participant's recommended fiber intake based on his or her reported energy intake. Average daily energy intake was divided by 1,000 and this quotient was multiplied by 14. Individuals with a total fiber consumption greater than this value were categorized as meeting AI recommendations, and individuals with a total fiber consumption less than this value were categorized as not meeting AI recommendations. Using the 14 grams per 1,000 kilocalorie intake recommendation had two advantages. First, it was independent of age and sex. Second, this method circumvented the underreporting limitation inherent to

food frequency questionnaires by determining AI fiber intake from within the dataset. **Statistical Analyses**

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corp., Armonk, NY, 2016). Alpha was set *a priori* at 0.05. Descriptive statistics were conducted to determine means, medians, and standard deviations of each variable. Assumptions of normality were tested with Kolmogorov-Smirnov (K-S) tests. Dietary intake variables were found to violate assumptions of normality, thus nonparametric Spearman's *rho* correlation coefficients and partial Spearman's correlation coefficients were calculated. Mann-Whitney U tests were conducted to determine sex differences between participant characteristics and intake variables identified as having non-normal distributions. Chi-square tests were applied to the relationship between men and women meeting nutrient needs for fiber, between men and women meeting nutrient needs for calcium, between men and women meeting nutrient needs for vitamin D, and between men and women meeting both calcium and fiber needs. Independent sample t-tests were conducted to determine sex differences between variables identified as having a normal distribution. These descriptive analyses were repeated in the subset of participants for whom accelerometer data were available to determine differences between these variables among participants in the lower quartile of time spent in moderate and vigorous physical activity

versus the upper quartile of time spent in moderate and vigorous physical activity.

**RESULTS**

Data from 228 participants were evaluated for inclusion in analyses. **Figure 1** details the processes by which 95 participants were included for data analyses. A total of 133 participants were excluded from analyses for lacking status as an endurance athlete, missing data, reporting medication

*Tests for Normality*

Results of the K-S test for distributions among the overall sample and among men and women are provided in **Table 1** and **Table 2**, respectively.

*Participant Characteristics*

Descriptive characteristics may be found in **Tables 4 and 5**. Male participants had significantly higher values for height ($t(93) = 9.78$, $p < 0.001$), fat-free mass ($t(77.38) = 15.57$, $p < 0.001$), energy intake ($U = 792.50$, $p = 0.013$), and adequate intake recommendations for total fiber intake ($U = 792.50$, $p = 0.013$). There were no significant differences between the sexes for age; lumbar spine BMD; body mass index; or protein as percent energy, total fiber, soluble fiber, total calcium, or total vitamin D intakes. Calcium and vitamin D intakes represent combined dietary and supplementation intakes. Dietary vitamin D intake was included because this intake variable was significantly correlated

with AP lumbar spine BMD ($r_s(93) = 0.249$, $p = 0.015$). Dietary calcium intake was not significantly correlated with AP lumbar spine BMD.

Frequencies representing the number of participants meeting Adequate Intake (AI) guidelines for total fiber, the number of participants meeting Recommended Dietary Allowance (RDA) guidelines each for calcium and vitamin D, and the number of participants meeting both AI recommendations for fiber intake and RDA guidelines for calcium intake may be found in **Table 6**. Most participants failed to meet AI guidelines for fiber intake, with only 22% of participants meeting fiber intake guidelines. Less than half (48%) of participants met the RDA for calcium intake, and less than 12% of participants met the RDA for vitamin D. Only 12 out of 95 participants (12.6%) met both intake recommendations for calcium and fiber. Chi-square tests were applied to the relationship between men and women meeting nutrient needs for total fiber intake ($\chi_2(1, N = 95) = 2.810$, $p = 0.094$), between men and women meeting nutrient needs for calcium ($\chi_2(1, N = 95) = 1.772$, $p = 0.183$), between men and women meeting nutrient needs for vitamin D ($\chi_2(1, N = 95) = 2.453$, $p = 0.117$), and between men and women meeting both calcium and fiber needs ($\chi_2(1, N = 95) = 0.335$, $p = 0.563$). No significant sex differences were found, indicating that both men and women had a statistically equal likelihood for failing to meet these nutrient needs.

***Associations between Dietary Intake Variables and AP Lumbar Spine BMD***

All correlations among dietary intake variables, fat-free mass, body mass index, and AP lumbar spine BMD are listed in **Table 7.** No significant relationship was found between soluble fiber intake and AP lumbar spine BMD among any of the groups. Although none of the relationships were significant, all correlation coefficients calculated for soluble fiber intake and lumbar spine BMD were negative. No significant relationships were found between total fiber intake and AP lumbar spine BMD among any of the groups.

Intakes of total calcium, total vitamin D, and overall energy intake were not found to be significantly related to AP lumbar spine BMD. Interestingly, protein as percent of total energy intake was found to be significantly correlated with AP lumbar spine BMD for the population subset who met AI recommendations for fiber intake ($r_s(19) = 0.571$, $p = 0.007$). Body mass index (BMI) was significantly positively correlated with AP lumbar BMD in both the overall sample ($r_s(93) = 0.275$, $p = 0.007$) as well as among female participants ($r_s(46) = 0.330$, $p = 0.022$). The correlation coefficient for BMI and AP lumbar spine BMD was comparable to the correlation coefficient found in the overall sample ($r_s(45) = 0.263$, $p = 0.074$). All three of these findings suggest a weak positive relationship between BMI and AP lumbar spine BMD. A positive significant correlation was found between fat-free mass and AP lumbar spine

BMD in the overall sample ($r(93) = 0.273$, $p = 0.008$) and in the sample of female participants ($r(46) = 0.293$, $p = 0.043$).

A partial Spearman's correlation coefficient was calculated between soluble fiber intake and AP lumbar spine BMD to control for the influence of fat-free mass, dietary vitamin D intake and BMI. No significant partial Spearman's correlation coefficient was found between soluble fiber and AP lumbar spine BMD ($r_s(91) = -0.080$, $p = 0.447$). No significant partial Spearman's correlation coefficient was found between total fiber intake and AP lumbar spine BMD ($r_s(91) = -0.127$, $p = 0.226$).

*Between Group Differences*

A z-test was performed to assess between-group differences for sex, calcium intake, fiber intake, and time spent in combined moderate and vigorous physical activity. To determine sex differences, a regression equation was generated for the relationship between soluble fiber intake and bone mineral density for male participants and female participants. The z-test yielded a value of 0.02, indicating that there was no statistically significant difference between the slopes because this value fell within the ±1.96 range. The z-test value for the difference in slopes between participants who met AI recommendations for fiber and participants who did not meet AI recommendations for fiber was zero, indicating no difference. No significant differences were found between groups

for meeting versus not meeting the RDA for calcium (z = -0.188). No significant differences were found between the upper and lower quartiles of time spent in combined moderate and vigorous physical activity (z = -0.82).

**DISCUSSION**

The relationship between total and soluble fiber intakes and anterior-posterior lumbar spine bone mineral density were assessed in 95 endurance athletes who participated in the ongoing cross-sectional Drexel Fitness Study. No relationship was identified between soluble or total fiber intake and anterior-posterior lumbar spine bone mineral density in this sample of athletes. Although researchers have reported that increased soluble fiber intake correlated positively with calcium absorption in adolescents[14,15] and was associated with decreased rates of bone loss in older adults[26], it remains to be seen whether these findings may be translated into a population of healthy adults.

We were unable to measure biomarkers associated with calcium and bone metabolism, such as parathyroid hormone, cross-linked N-telopeptides of type I collagen, or bone alkaline phosphatase that may have provided additional insight about the bone health of our participants. Calcium metabolism is highly regulated, and transcellular absorption is upregulated during periods of low intake or increased demand.[27] Transcellular calcium absorption may be upregulated in athletes following bouts of heavy sweating to compensate for

losses during physical activity. Therefore, while calcium absorption may be augmented by the fermentation of fibers in the large intestine, this may not be clinically relevant to bone health or exert long-term changes on bone mineral density. Indeed, researchers found no significant changes to biomarkers associated with calcium and bone metabolism following soluble fiber supplementation.[14,15] Our findings suggest that soluble fiber and total fiber intakes may not play a role in enhancing bone density among healthy endurance athletes. Nonetheless, this was a cross-sectional study, which limits our conclusions.

Another mechanism by which fiber is proposed to promote bone health is through the mitigation of pro-inflammatory responses induced by prolonged physical activity. Although extremely high volumes of endurance training have been shown to be detrimental to bone health[10,11,28], we did not observe a detrimental relationship between activity level and AP lumbar spine BMD in the population of athletes sampled for our analyses. Although all of the athletes included for analyses specified endurance activity as the athlete's primary sport, two were professional athletes, while a majority of participants (60%) were recreational athletes. The extreme high level of physical activity demanded of the professional athletes in which bone loss is observed was probably not the usual workout regimen of most participants included in this analysis. Indeed,

the median time spent in vigorous activity was 227 minutes for the seven days for which accelerometers were worn, or 3.78 hours. The mean number of reported days of exercise per week was 5.29 ± 1.17 days.

Although participants exceeded the American College of Sports Medicine's physical activity guideline recommendations of 150 minutes per week of moderate intensity exercise[23], their physical activity level is lower than that of professional athletes. Whitfield and colleagues presented similar findings in their analysis of physical activity reported in NHANES data.[29] Reduced BMD was not observed among men or women exceeding physical activity guidelines. The researchers asserted that this may have occurred because the self-reported activity was not comparable to the energy expenditure of professional athletes with large training volumes.[29]

Given these findings, directions for future research may re-evaluate the relationship between fiber and bone health among athletes with larger training volumes. In the meantime, the key take-away from our current analyses is the alarmingly low consumption of fiber observed in this cohort of athletes. A mere 21 out of 95 athletes reported intakes that met the Adequate Intake recommendations for fiber intake. While the relationship between fiber and BMD may be moot in our study, fiber has many well-documented health benefits.[25] Furthermore, fiber may serve as a "canary in the coal mine",

indicating that participants' diets may also be lacking in fiber-rich fruits and vegetables. Dietary recommendations should consider emphasizing fiber outside of competition times to help resolve this gap.

**STRENGTHS AND LIMITATIONS**

A major strength of this study was the use of DXA to measure bone mineral density. Aside from being a non-invasive and reliable measure, DXA is a clinical gold standard for measuring bone mineral density. The availability of DXA body composition to obtain fat-free mass as a covariate is another strength. Second, our request that participants disclose any medications they were currently prescribed is another strength, because this information allowed us to exclude participants whose BMD may have been confounded by medication use. Third, the Food Frequency Questionnaire used in this study estimates yearly intakes. This is an important consideration when correlating dietary intake variables to BMD. Bone mineral density is a long-term indicator of bone health, and dietary recall methods that consider food intake for less than one year may not capture the long-term effects of diet on bone. Another strength was the recruitment of a diverse population of healthy adult athletes, which increased the external validity of the study.

Several limitations proved detrimental to our analyses. First, we did not record the frequency of medication use. Therefore, participants who used an

asthma inhaler intermittently may have been unnecessarily excluded from analyses. Second, the ubiquitous use of oral contraceptives is a three-fold limitation. First, because researchers have observed inconsistent effects of oral contraceptives on bone mineral density, rendering it impossible to determine its effects.[30] Second, the reason participants take oral contraceptives is unclear in our data collection procedures, and participants taking it to "cure" oligomenorrhea or amenorrhea may have underlying medical issues that could affect bone mineral density. Third, the ubiquitous use of oral contraceptives made it impossible for us to exclude participants reporting its use from our analyses. Foremost, the sample size would be crippled. Additionally, the distribution of men and women would have been tremendously skewed.

The FFQ, although a strength, in terms of estimating yearly intake, has several disadvantages. The questionnaire lacks a category for probiotic supplementation, and probiotics may alter the gut microbiome's response to fiber. The FFQ also fails to distinguish between different types of supplements. This is particularly problematic for calcium and vitamin D, because there are vast differences in the bioavailability of different forms of calcium and between vitamin $D_2$ and vitamin $D_3$. The FFQ also has limited options for alternatives to dairy products, and participants frequently complained about the lack of options. Therefore, calcium specifically may be underreported. Although the

FFQ provides an estimate of dietary and supplemental vitamin D intake, serum calcidiol concentrations were not measured and sun exposure was not assessed. Thus, another limitation was that the participants' vitamin D status was unknown.

A final limitation is the lack of data on long-term training history. Childhood physical activity has been shown to be an important predictor of bone health across the lifespan,[31] and we lack the data to account for this. Additionally, the human body resiliently adapts to regular training. Training upregulates pathways involved in hyperthermia adaptation and antioxidant defense.[32] Well-trained athletes have higher than normal concentrations of anti-lipopolysaccharide immunoglobulins, indicating an adaptive response to repeated lipopolysaccharide exposure.[33] Researchers have also reported that training status increases mRNA expression of glucocorticoid receptor-alpha, suggesting that increased training status enhances the body's ability to clear cortisol from plasma.[34] These findings indicate that well-trained athletes may adapt to the stressors that are detrimental to bone health, mitigating the role of fermentable fibers in protecting bone. We lacked the ability to distinguish between highly-trained athletes and athletes with less training experience in this cross-sectional study.

## CONCLUSIONS

The correlations derived from this cross-sectional data analysis hardly serve as a benchmark for resolving the lack of research in this area. The data we presented here seem to indicate that fiber intake and bone mineral density are not related. More research is required to determine whether fiber plays any sort of meaningful role in bone health in healthy adults and athletes.

## TABLES

**Table 1. Kolmogorov-Smirnov Test for Normality: Results of Overall Data**

| Variable | Statistic | Degrees of freedom | *p*-value |
|---|---|---|---|
| Age in years | 0.114 | 95 | 0.004 |
| Height (cm) | 0.049 | 95 | 0.200* |
| Weight (kg) | 0.099 | 95 | 0.022 |
| Body mass index (kg/m$^2$) | 0.168 | 95 | < 0.001 |
| Fat free mass (kg) | 0.103 | 95 | 0.015 |
| Lumbar bone mineral density (g/cm$^2$) | 0.064 | 95 | 0.200* |
| Energy intake (kilocalories) | 0.113 | 95 | 0.005 |
| Protein intake (g) | 0.118 | 95 | 0.002 |
| Total fiber intake (g) | 0.116 | 95 | 0.003 |
| Soluble fiber intake (g) | 0.123 | 95 | 0.001 |
| Total calcium intake (mg) | 0.142 | 95 | < 0.001 |
| Total vitamin D intake (IU) | 0.159 | 95 | < 0.001 |
| Protein as percent of energy | 0.107 | 95 | 0.009 |
| Individualized fiber adequate intake (g) | 0.113 | 95 | 0.005 |
| Time (minutes) in moderate/vigorous activity | 0.119 | 55 | < 0.001 |

* $p > 0.05$ indicates normal distribution of data

cm = centimeters; kg = kilograms; m$^2$ = meters squared; g = grams; mg = milligrams; IU = international units

**Table 2. Kolmogorov-Smirnov Test for Normality: Results by Sex**

| Variable | Sex | Statistic | Degrees of freedom | *p* value |
|---|---|---|---|---|
| Age in years | male | 0.166 | 47 | 0.002 |
| | female | 0.174 | 48 | 0.001 |
| Height (cm) | male | 0.079 | 47 | 0.200* |
| | female | 0.081 | 48 | 0.200* |
| Weight (kg) | male | 0.137 | 47 | 0.028 |
| | female | 0.139 | 48 | 0.020 |
| Body mass index (kg/m$^2$) | male | 0.206 | 47 | < 0.001 |
| | female | 0.188 | 48 | < 0.000 |
| Fat free mass (kg) | male | 0.111 | 47 | 0.193 |
| | female | 0.067 | 48 | 0.200* |
| Lumbar bone mineral density (g/cm$^2$) | male | 0.084 | 47 | 0.200* |
| | female | 0.072 | 48 | 0.200* |
| Energy intake (kilocalories) | male | 0.140 | 47 | 0.022 |
| | female | 0.150 | 48 | 0.009 |
| Individualized fiber adequate intake | male | 0.140 | 47 | 0.022 |
| | female | 0.150 | 48 | 0.009 |
| Total fiber intake (g) | male | 0.131 | 47 | 0.044 |
| | female | 0.100 | 48 | 0.200* |
| Soluble fiber intake (g) | male | 0.134 | 47 | 0.035 |
| | female | 0.106 | 48 | 0.200* |
| Protein as percent of energy | male | 0.152 | 47 | 0.008 |
| | female | 0.084 | 48 | 0.200* |
| Total calcium intake (mg) | male | 0.145 | 47 | 0.014 |
| | female | 0.167 | 48 | 0.002 |
| Total vitamin D intake (IU) | male | 0.186 | 47 | 0.000 |
| | female | 0.141 | 48 | 0.019 |
| Protein intake (g) | male | 0.108 | 47 | 0.200* |
| | female | 0.180 | 48 | 0.000 |
| Individualized fiber adequate intake (g) | male | | | |
| | female | | | |
| Time (minutes) in moderate/vigorous activity | male | 0.277 | 27 | < 0.001 |
| | female | 0.187 | 28 | 0.014 |

*p* > 0.05 indicates normal distribution of data. cm = centimeters; kg = kilograms; m$^2$ = meters squared; g = grams; mg = milligrams; IU = international units

**Table 3. Kolmogorov-Smirnov Test for Normality: Results by Time Spent in Moderate and Vigorous Physical Activity**

| Variable | Quartile | Statistic | Degrees of freedom | *p* value |
|---|---|---|---|---|
| Age in years | lower | 0.140 | 27 | 0.186* |
| | upper | 0.151 | 28 | 0.099* |
| Height (cm) | lower | 0.099 | 27 | 0.200* |
| | upper | 0.122 | 28 | 0.200* |
| Weight (kg) | lower | 0.150 | 27 | 0.124* |
| | upper | 0.112 | 28 | 0.200* |
| Body mass index (kg/m$^2$) | lower | 0.262 | 27 | < 0.001 |
| | upper | 0.194 | 28 | 0.008 |
| Fat free mass (kg) | lower | 0.193 | 27 | 0.011 |
| | upper | 0.081 | 28 | 0.200* |
| Lumbar bone mineral density (g/cm2) | lower | 0.154 | 27 | 0.102* |
| | upper | 0.144 | 28 | .141 |
| Energy intake (kilocalories) | lower | 0.175 | 27 | 0.033 |
| | upper | 0.164 | 28 | 0.051* |
| Individualized fiber adequate intake | lower | 0.175 | 27 | 0.033 |
| | upper | 0.164 | 28 | 0.051* |
| Total fiber intake (g) | lower | 0.202 | 27 | 0.006 |
| | upper | 0.095 | 28 | 0.200* |
| Soluble fiber intake (g) | lower | 0.187 | 27 | 0.016 |
| | upper | 0.112 | 28 | 0.200* |
| Protein as percent of energy | lower | 0.119 | 27 | 0.200* |
| | upper | 0.175 | 28 | 0.028 |
| Total calcium intake (mg) | lower | 0.168 | 27 | 0.048 |
| | upper | 0.203 | 28 | 0.004 |
| Total vitamin D intake (IU) | lower | 0.170 | 27 | 0.043 |
| | upper | 0.164 | 28 | 0.051* |
| Protein intake (g) | lower | 0.215 | 27 | 0.002 |
| | upper | 0.162 | 28 | 0.058* |

* $p > 0.05$ indicates normal distribution of data

cm = centimeters; kg = kilograms; m$^2$ = meters squared; g = grams; mg = milligrams; IU = international units

**Table 4. Descriptive Statistics of Participant Characteristics (Normally Distributed Data)**

| Characteristic | Total Sample n = 95 Mean ± SD | Male Participants n = 47 Mean ± SD | Female Participants n = 48 Mean ± SD |
|---|---|---|---|
| Age (years) | 38.15 ± 10.07 | 36.60 ± 10.52 | 39.67 ± 9.47 |
| Height (cm) | 172.21 ± 10.69 | 179.86 ± 7.72** | 164.72 ± 7.38 |
| Fat-free mass (kg) | 54.60 ± 10.87 | 63.90 ± 6.87** | 45.49 ± 4.34 |
| Lumbar BMD ($g/cm^2$) | 1.26 ± 0.15 | 1.29 ± 0.14 | 1.24 ± 0.16 |

SD = standard deviation; cm = centimeters; kg = kilograms; BMD = bone mineral density; g = grams; $cm^2$ = centimeters squared

**$p < 0.05$, value significantly higher than opposite sex

**Table 5. Descriptive Statistics of Participant Characteristics (Non-normally Distributed Data)**

| Characteristic | Total Sample n = 95 Median | Male Participants n = 47 Median | Female Participants n = 48 Median |
|---|---|---|---|
| Weight (kg) | 71.60 | 76.45 | 64.29 |
| Body mass index ($kg/m^2$) | 23.68 | 24.38 | 23.51 |
| Energy intake (kcal) | 1815.45 | 2069.77** | 1659.87 |
| Protein as percent total energy | 15.25 | 15.25 | 15.15 |
| Total fiber intake (g) | 21.03 | 20.11 | 21.25 |
| Soluble fiber intake (g) | 6.73 | 6.91 | 6.58 |
| Fiber Adequate Intake (g) | 25.41 | 28.98** | 23.24 |
| Total calcium intake (mg) | 986.94 | 1023.26 | 908.54 |
| Total vitamin D intake (IU) | 264.21 | 190.11 | 273.63 |

kg = kilograms; $m^2$ = meters squared; kcal = kilocalories; g = grams; mg = milligrams; IU = international units

**$p < 0.05$, value significantly higher than opposite sex

**Table 6. Frequency of Participants Meeting Nutrient Needs**

| Nutrient | Total Sample (n = 95) | Male Participants (n = 47) | Female Participants (n = 48) |
|---|---|---|---|
| Total fiber | 21 | 7 | 14 |
| Calcium | 46 | 26 | 20 |
| Vitamin D | 11 | 3 | 8 |
| Total fiber and calcium | 12 | 5 | 7 |

Total fiber needs based on Adequate Intake recommendation of 14 grams per 1,000 kilocalories; calcium needs based on 1,300 milligrams (mg) per day Recommended Dietary Allowance (RDA) for individuals 14 to 18 years of age, 1,000 mg per day for individuals 19 to 50 years of age, 1,000 mg per day for men 51 to 70 years of age, 1,200 mg per day for women 51 to 70 years of age; vitamin D needs based on RDA of 600 International Units per day for individuals 1 to 70 years of age.

**Table 7. Spearman Correlation Coefficients between Dietary Intake Variables, Body Mass Index, Fat-Free Mass, and Anterior-Posterior Lumbar Spine Bone Mineral Density**

| Factor | Lumbar BMD ($g/cm^2$) | Soluble Fiber (g) | Total Fiber (g) | Calcium (mg) | Vitamin D (IU) | Protein (%kcal) | Energy (kcal) | Fat-free Mass (kg) | BMI ($kg/m^2$) |
|---|---|---|---|---|---|---|---|---|---|
| **1. AP Lumbar BMD ($g/cm^2$)** | | | | | | | | | |
| Total Sample (n = 95) | 1.000 | -0.085 | -0.138 | -0.024 | 0.143 | 0.113 | 0.018 | 0.257 ** | 0.275 ** |
| Male Participants (n = 47) | 1.000 | -0.112 | -0.120 | -0.010 | 0.197 | 0.034 | -0.006 | 0.252 ** *** | 0.263 |
| Female Participants (n = 48) | 1.000 | -0.090 | -0.143 | -0.064 | 0.077 | 0.166 | 0.006 | 0.293 ** *** | 0.330 ** |
| Met Fiber AI (n = 21) | 1.000 | -0.104 | -0.184 | 0.006 | 0.190 | 0.571 ** | -0.023 | 0.437 | 0.382 |
| Did Not Meet Fiber AI (n = 74) | 1.000 | -0.037 | -0.093 | -0.036 | 0.114 | -0.024 | 0.021 | 0.227 | 0.240 ** |
| Met Calcium RDA (n = 46) | 1.000 | -0.089 | -0.140 | -0.094 | 0.152 | 0.125 | 0.147 | 0.257 | 0.163 |
| Did Not Meet Calcium RDA (n = 49) | 1.000 | -0.077 | -0.154 | -0.157 | 0.130 | 0.089 | -0.128 | 0.246 | 0.381 ** |
| Upper Quartile of Activity (n = 28) | 1.000 | -0.001 | 0.001 | -0.122 | 0.161 | 0.191 | -0.004 | 0.135 | 0.504 ** |
| Lower Quartile of Activity (n = 27) | 1.000 | -0.019 | -0.136 | 0.101 | 0.224 | 0.159 | 0.048 | 0.304 | 0.503 ** |
| **2. Soluble Fiber (g)** | | | | | | | | | |
| Total Sample | -0.085 | 1.000 | 0.975 ** | 0.493 ** | -0.061 | -0.216 | 0.724 | 0.029 | -0.073 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (n = 95) | | | | | | | | | |
| Male Participants (n = 47) | -0.112 | 1.000 | 0.970 ** | 0.642 ** | -0.126 | 0.021 | 0.720 ** | 0.038 | -0.210 |
| Female Participants (n = 48) | -0.090 | 1.000 | 0.975 ** | 0.328 ** | 0.016 | -0.444 ** | 0.716 ** | -0.128 | 0.017 |
| Met Fiber AI (n = 21) | -0.104 | 1.000 | 0.956 ** | 0.116 | -0.316 | -0.209 | 0.947 ** | 0.344 | -0.031 |
| Did Not Meet Fiber AI (n = 74) | -0.037 | 1.000 | 0.968 | 0.566 | -0.026 | -0.183 | 0.836 | 0.105 | -0.032 |
| Met Calcium RDA (n = 46) | -0.089 | 1.000 | 0.961 ** | 0.014 | -0.331 ** | -0.146 | 0.579 ** | 0.130 | -0.206 |
| Did Not Meet Calcium RDA (n = 49) | -0.077 | 1.000 | 0.969 ** | 0.360 ** | -0.382 ** | -0.333 ** | 0.698 ** | -0.145 | 0.014 |
| Upper Quartile of Activity (n = 28) | -0.001 | 1.000 | 0.974 ** | 0.696 ** | -0.115 | -0.294 | 0.818 ** | 0.303 | 0.143 |
| Lower Quartile of Activity (n = 27) | -0.019 | 1.000 | 0.964 ** | 0.074 | -0.239 | -0.511 ** | 0.668 ** | -0.250 | -0.167 |
| **3. Total Fiber (g)** | | | | | | | | | |
| Total Sample (n = 95) | -0.138 | 0.975 ** | 1.000 | 0.489 ** | -0.059 | -0.187 | 0.685 ** | 0.009 | -0.093 |
| Male Participants (n = 47) | -0.120 | 0.970 ** | 1.000 | 0.607 ** | -0.131 | 0.063 | 0.684 ** | 0.111 | -0.188 |
| Female Participants (n = 48) | -0.143 | 0.975 ** | 1.000 | 0.352 ** | 0.046 | -0.436 ** | 0.687 ** | -0.170 | -0.009 |
| Met Fiber AI (n = 21) | -0.184 | 0.956 ** | 1.000 | 0.126 | -0.300 | -0.219 | 0.945 ** | 0.277 | -0.057 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Did Not Meet Fiber AI (n = 74) | -0.093 | 0.968 ** | 1.000 | 0.555 ** | -0.041 | -0.131 | 0.794 ** | 0.103 | -0.030 |
| Met Calcium RDA (n = 46) | -0.140 | 0.961 ** | 1.000 | 0.106 | -0.285 | -0.141 | 0.543 ** | 0.109 | -0.244 |
| Did Not Meet Calcium RDA (n = 49) | -0.154 | 0.969 ** | 1.000 | 0.398 ** | -0.377 ** | -0.291 ** | 0.679 ** | -0.137 | -0.027 |
| Upper Quartile of Activity (n = 28) | 0.001 | 0.974 *8 | 1.000 | 0.732 ** | -0.063 | -0.345 | 0.814 ** | 0.342 | 0.116 |
| Lower Quartile of Activity (n = 27) | -0.136 | 0.964 ** | 1.000 | 0.115 | -0.225 | -0.424 ** | 0.551 ** | -0.336 | -0.236 |
| **4. Calcium (mg)** | | | | | | | | | |
| Total Sample (n = 95) | -0.024 | 0.493 | 0.489 ** | 1.000 | 0.564 ** | 0.128 | 0.543 ** | 0.050 | -0.115 |
| Male Participants (n = 47) | -0.010 | 0.642 ** | 0.607 ** | 1.000 | 0.402 ** | 0.225 | 0.826 ** | 0.135 | -0.322 ** |
| Female Participants (n = 48) | -0.064 | 0.328 ** | 0.352 ** | 1.000 | 0.727 ** | 0.045 | 0.209 | -0.112 | 0.051 |
| Met Fiber AI (n = 21) | 0.006 | 0.116 | 0.126 | 1.000 | 0.819 ** | 0.087 | 0.156 | -0.169 | 0.184 |
| Did Not Meet Fiber AI (n = 74) | -0.036 | 0.566 ** | 0.555 ** | 1.000 | 0.499 ** | 0.126 | 0.644 ** | 0.094 | -0.176 |
| Met Calcium RDA (n = 46) | -0.094 | 0.014 | 0.106 | 1.000 | 0.401 ** | -0.031 | 0.052 | -0.196 | -0.379 ** |
| Did Not Meet Calcium RDA | -0.157 | 0.360 ** | 0.398 ** | 1.000 | 0.277 | 0.236 | 0.532 ** | -0.061 | -0.130 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (n = 49) | | | | | | | | | |
| Upper Quartile of Activity (n = 28) | -0.122 | 0.696 ** | 0.732 ** | 1.000 | 0.473 ** | -0.180 | 0.701 | 0.210 | 0.032 |
| Lower Quartile of Activity (n = 27) | 0.101 | 0.074 | 0.115 | 1.000 | 0.728 ** | 0.501 ** | 0.143 | -0.009 | -0.103 |
| **5. Vitamin D (IU)** | | | | | | | | | |
| Total Sample (n = 95) | 0.143 | -0.061 | -0.059 | 0.564 ** | 1.000 | 0.346 ** | -0.016 | -0.093 | -0.074 |
| Male Participants (n = 47) | 0.197 | -0.126 | -0.131 | 0.402 ** | 1.000 | 0.525 ** | 0.084 | 0.108 | -0.139 |
| Female Participants (n = 48) | 0.077 | 0.016 | 0.046 | 0.727 ** | 1.000 | 0.179 | -0.105 | -0.127 | 0.001 |
| Met Fiber AI (n = 21) | 0.190 | -0.316 | -0.300 | 0.819 ** | 1.000 | 0.299 | -0.230 | -0.266 | 0.231 |
| Did Not Meet Fiber AI (n = 74) | 0.114 | -0.026 | -0.041 | 0.499 ** | 1.000 | 0.341 ** | 0.062 | -0.051 | -0.200 |
| Met Calcium RDA (n = 46) | 0.152 | -0.331 ** | -0.285 | 0.401 ** | 1.000 | 0.246 | -0.371 ** | -0.278 | -0.038 |
| Did Not Meet Calcium RDA (n = 49) | 0.130 | -0.382 ** | -0.377 ** | 0.277 | 1.000 | 0.451 ** | -0.260 | -0.065 | -0.086 |
| Upper Quartile of Activity (n = 28) | 0.161 | -0.115 | -0.063 | 0.473 ** | 1.000 | 0.411 ** | -0.016 | -0.146 | -0.151 |
| Lower Quartile of Activity (n = 27) | 0.224 | -0.239 | -0.225 | 0.728 ** | 1.000 | 0.582 ** | -0.170 | 0.073 | -0.046 |
| **6. Protein as Percent Energy** | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total Sample (n = 95) | 0.113 | -0.216** | -0.187 | 0.128 | 0.346** | 1.000 | -0.130 | 0.087 | 0.027 |
| Male Participants (n = 47) | 0.034 | 0.021 | 0.063 | 0.225 | 0.525** | 1.000 | 0.034 | 0.281 | 0.124 |
| Female Participants (n = 48) | 0.166 | -0.444** | -0.436** | 0.045 | 0.179 | 1.000 | -0.338** | 0.148 | 0.040 |
| Met Fiber AI (n = 21) | 0.571** | -0.209 | -0.219 | 0.087 | 0.299 | 1.000 | -0.100 | 0.255 | 0.095 |
| Did Not Meet Fiber AI (n = 74) | -0.024 | -0.183 | -0.131 | 0.126 | 0.341** | 1.000 | -0.174 | 0.003 | -0.006 |
| Met Calcium RDA (n = 46) | 0.125 | -0.146 | -0.141 | -0.031 | 0.246 | 1.000 | -0.186 | 0.158 | 0.163 |
| Did Not Meet Calcium RDA (n = 49) | 0.089 | -0.333** | -0.291** | 0.236 | 0.451** | 1.000 | -0.162 | -0.006 | -0.104 |
| Upper Quartile of Activity (n = 28) | 0.191 | -0.294 | -0.345 | -0.180 | 0.411** | 1.000 | -0.252 | -0.125 | 0.089 |
| Lower Quartile of Activity (n = 27) | 0.159 | -0.511** | -0.424** | 0.501** | 0.582** | 1.000 | -0.437** | 0.302 | 0.032 |
| **7. Energy Intake (kcal)** | | | | | | | | | |
| Total Sample (n = 95) | 0.018 | 0.724** | 0.685 | 0.543** | -0.016 | -0.130 | 1.000 | 0.238** | -0.077 |
| Male Participants (n = 47) | -0.006 | 0.720** | 0.684** | 0.826** | 0.084 | 0.034 | 1.000 | 0.201 | -0.211 |
| Female Participants (n = 48) | 0.006 | 0.716** | 0.687** | 0.209 | -0.105 | -0.338** | 1.000 | -0.119 | -0.003 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Met Fiber AI (n = 21) | 0-.023 | 0.947 ** | 0.945 ** | 0.156 | -0.230 | -0.100 | 1.000 | 0.294 | 0.027 |
| Did Not Meet Fiber AI (n = 74) | 0.021 | 0.836 ** | 0.794 ** | 0.644 ** | 0.062 | -0.174 | 1.000 | 0.195 | -0.104 |
| Met Calcium RDA (n = 46) | 0.147 | 0.579 ** | 0.543 ** | 0.052 | -0.371 ** | -0.186 | 1.000 | 0.500 ** | -0.286 |
| Did Not Meet Calcium RDA (n = 49) | -0.128 | 0.698 ** | 0.679 ** | 0.532 ** | -0.260 | -0.162 | 1.000 | -0.165 | -0.003 |
| Upper Quartile of Activity (n = 28) | -0.004 | 0.818 ** | 0.814 ** | 0.701 ** | -0.016 | -0.252 | 1.000 | 0.539 ** | 0.108 |
| Lower Quartile of Activity (n = 27) | 0.048 | 0.668 ** | 0.551 ** | 0.143 | -0.170 | -0.437 ** | 1.000 | -0.183 | -0.286 |
| **8. Fat-Free Mass (kg)** | | | | | | | | | |
| Total Sample (n = 95) | 0.257 ** | 0.029 | 0.009 | 0.050 | -0.093 | 0.087 | 0.238 ** | 1.000 | 0.253 ** |
| Male Participants (n = 47) | 0.276 | 0.038 | 0.111 | 0.135 | 0.108 | 0.281 | 0.201 | 1.000 | 0.285 |
| Female Participants (n = 48) | 0.301 ** | -0.128 | -0.170 | -0.112 | -0.127 | 0.148 | -0.119 | 1.000 | 0.404 ** |
| Met Fiber AI (n = 21) | 0.437 ** | 0.344 | 0.277 | -0.169 | -0.266 | 0.255 | 0.294 | 1.000 | 0.122 |
| Did Not Meet Fiber AI (n = 74) | 0.227 | 0.105 | 0.103 | 0.094 | -0.051 | 0.003 | 0.195 | 1.000 | 0.273 ** |
| Met Calcium RDA (n = 46) | 0.257 | 0.130 | 0.109 | -0.196 | -0.278 | 0.158 | 0.500 ** | 1.000 | 0.140 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Did Not Meet Calcium RDA (n = 49) | 0.246 | -0.145 | -0.137 | -0.061 | -0.065 | -0.006 | -0.165 | 1.000 | 0.376 ** |
| Upper Quartile of Activity (n = 28) | 0.135 | 0.303 | 0.342 | 0.210 | -0.146 | -0.125 | 0.539 ** | 1.000 | 0.320 |
| Lower Quartile of Activity (n = 27) | 0.304 | -0.250 | -0.336 | -0.009 | 0.073 | 0.302 | -0.183 | 1.000 | 0.487 ** |
| **9. Body Mass Index (kg/m²)** | | | | | | | | | |
| Total Sample (n = 95) | 0.275 ** | -0.073 | -0.093 | -0.115 | -0.074 | 0.027 | -0.077 | 0.253 ** | 1.000 |
| Male Participants (n = 47) | 0.263 | -0.210 | -0.188 | -0.322 ** | -0.139 | 0.124 | -0.211 | 0.285 | 1.000 |
| Female Participants (n = 48) | 0.330 ** | 0.017 | -0.009 | 0.051 | 0.001 | 0.040 | -0.003 | 0.404 ** | 1.000 |
| Met Fiber AI (n = 21) | 0.382 | -0.031 | -0.057 | 0.184 | 0.231 | 0.095 | 0.027 | 0.122 | 1.000 |
| Did Not Meet Fiber AI (n = 74) | 0.240 ** | -0.032 | -0.030 | -0.176 | -0.200 | -0.006 | -0.104 | 0.273 | 1.000 |
| Met Calcium RDA (n = 46) | 0.163 | -0.206 | -0.244 | -0.379 ** | -0.038 | 0.163 | -0.286 | 0.140 | 1.000 |
| Did Not Meet Calcium RDA (n = 49) | 0.381 ** | 0.014 | -0.027 | -0.130 | -0.086 | -0.104 | -0.003 | 0.376 ** | 1.000 |
| Upper Quartile of Activity (n = 28) | 0.504 ** | 0.143 | 0.116 | 0.032 | -0.151 | 0.089 | 0.108 | 0.320 | 1.000 |
| Lower Quartile of Activity | 0.503 ** | -0.167 | -0.236 | -0.103 | -0.046 | 0.032 | -0.286 | 0.487 ** | 1.000 |

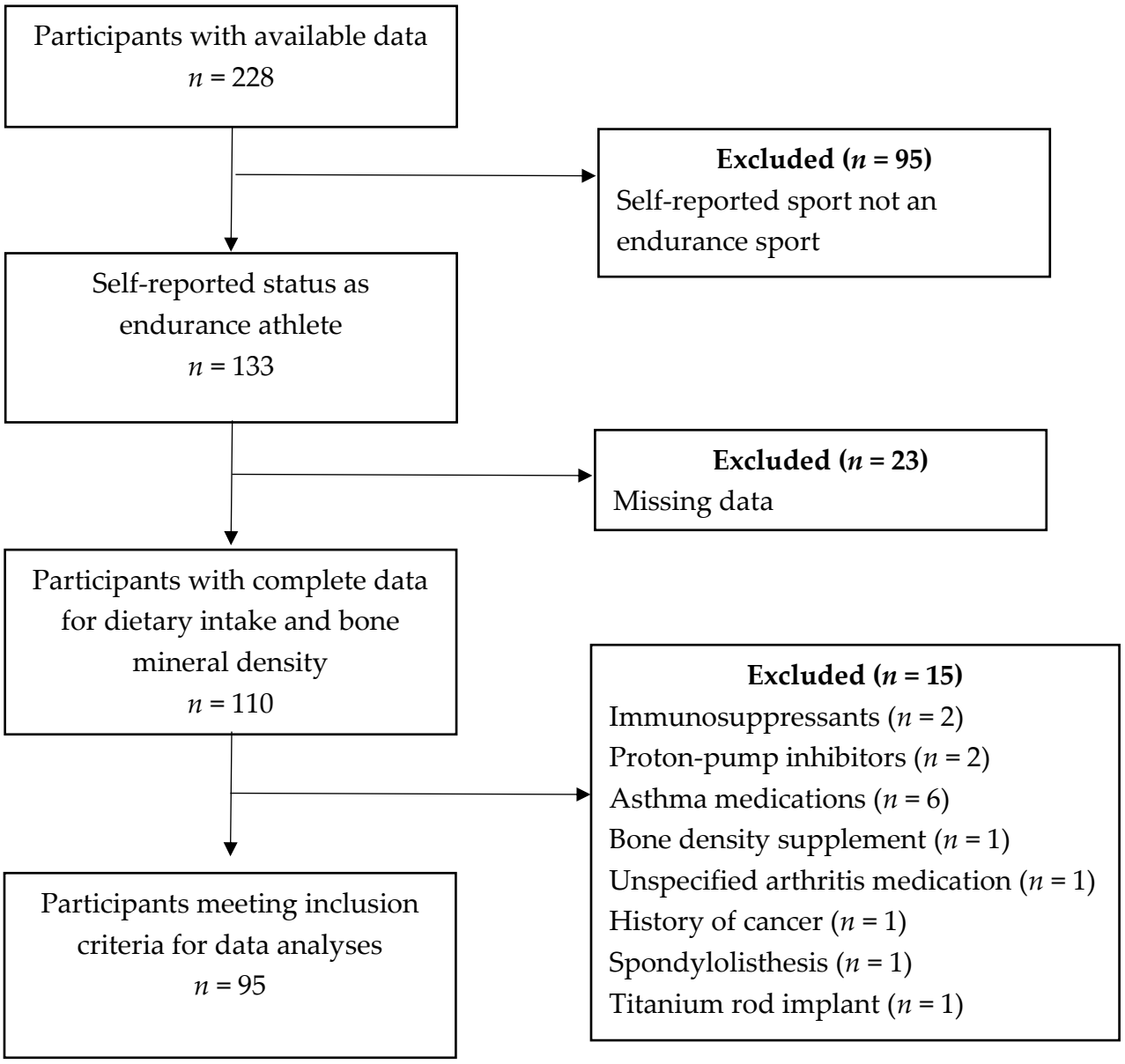
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (n = 27) | | | | | | | | | |

AP = anterior-posterior; BMD = bone mineral density; g = grams; $cm^2$ = centimeters squared; AI = Adequate Intake; RDA = Recommended Dietary Allowance; mg = milligrams; kcal = kilocalories; IU = international units; kg = kilograms; $m^2$ = meters squared;

**$p < 0.05$

***Pearson Product Moment Correlation coefficients were reported instead of Spearman's *rho* correlation coefficient because the data for both variables were normally distributed.

**Figure 1. Inclusion of Participants from Drexel Fitness Study**



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# APPENDICES

## APPENDIX A ~ Recruitment Flyer

# How fit are you??

**Find out in Drexel University's Nutrition Sciences' research study!**

**Are you a collegiate (club or recreational) athlete, an ROTC cadet or midshipman, or a Masters Athlete (age 26+), competitive or recreational?**

Dr. Stella Volpe's Metabolic Lab is looking for volunteers, **18 years of age or older, who exercise at least twice weekly**, to participate in a study where we will assess resting metabolic rate, body composition, aerobic capacity, and physical fitness **at no cost!**

- **Learn more about your own body composition!**
- **Find out your maximal oxygen consumption!**
- **Contribute to scientific understanding of your sport or program!**

For more information send email to:
**fitness.study@drexel.edu**
and use subject line "Nutrition Study"

APPROVED
Human Research Protection
Protocol # 1304002037A009
Approval Date: 7/11/16
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## APPENDIX B ~ Pre-Screening Survey Questions

### Qualtrics Pre-screen Survey for Inclusion into the Department of Nutrition Sciences Fitness Study

1. Please enter your first and last name. _____
2. What is your age? _____
3. What is your sex?
   a. Male
   b. Female
   c. Prefer not to answer
4. If female, are you currently or possibly pregnant?
   a. Yes
   b. No
   c. Prefer not to answer
   d. Not female
5. Do you smoke?
   a. Yes
   b. No
   c. Former
6. If formerly a smoker, how long has it been since you last smoked?
   a. Less than four months
   b. Less than six months
   c. Between six months and one year
   d. One year
   e. More than one year
7. What is the primary program or team to which you belong?
   a. Varsity Team
   b. Club Team
   c. ROTC Program
   d. Recreational/General Exercise
   e. Other (please specify): _____
8. What team are you a part of, or what is your primary form of exercise? (baseball, cycling, triathlete, CrossFit, etc…) Be as specific as possible:

   _____
9. On average, how many days per week do you exercise or does your team/program practice?
   a. 0 days
   b. 1 day

c. 2 days
d. 3 days
e. 4 days
f. 5 days
g. 6 days
h. 7 days

10. If you exercise/practice more than once a day, please note number of times per day you work-out, and number of days per week you work out more than one time per day.
    a. Yes (please describe):

       _____

    b. No, I only work-out once per day

11. Describe the intensity of your cardiovascular workouts.
    a. Easy
    b. Moderate
    c. Moderate-Intense
    d. High-Intensity
    e. Other (please specify): _____

12. Describe the intensity of your strength training workouts.
    a. Easy
    b. Moderate
    c. Moderate-Intense
    d. High-Intensity
    e. Other (please specify): _____

13. If you are a part of a team, do you exercise in addition to team/program workouts? If yes, please describe those other activities here. If you are also part of another team/program, please describe, as well
    a. Yes (please describe):

       _____

    b. No
    c. More than one team/program (please describe):

       _____

14. Have you been diagnosed, or are you aware of any pre-existing cardiac or other medical conditions that would affect your ability to participate in this study? (i.e. hypertension, cardiovascular disease, pregnancy, asthma, etc.) If yes, please describe.
    a. Yes (please list):

       _____

    b. No

## APPENDIX C ~ Informed Consent Document



ICF version: 3
Revision Date: October 30, 2013

**Drexel University**
**Consent to Take Part**
**In a Research Study**

***1. Title of research study:*** A Comparison of Fitness Characteristics in Collegiate Athletes, Reserve Officers' Training Corps (ROTC) Cadets and Midshipmen, and Masters Athletes

***2. Researcher:*** Stella L. Volpe, PhD, RD, LDN, FACSM

### *3. Why you are being invited to take part in a research study*

We invite you to take part in a research study because you are a collegiate athlete at the recreational, club, or varsity level, a Reserve Officers' Training Corps (ROTC) cadet or midshipman, or an active individual who is 26 years of age or older, also known as a "masters athlete."

### *4. What you should know about a research study*

- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

### *5. Who can I talk to?*

If you have questions, concerns, or complaints, or think the research has hurt you, talk to Dr. Stella L. Volpe at (267)-359-5826 or slv43@drexel.edu. A graduate student on the team can also be reached at (310)-663-9726 or jlh497@drexel.edu (Jody Herman).

This research has been reviewed and approved by an Institutional Review Board. You may talk to them at (215) 255-7857 or email HRPP@drexel.edu for any of the following:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

### *6. Why are we doing this research?*

The purpose of this study is to record and compare physical fitness characteristics of collegiate athletes, Reserve Officers' Training Corps (ROTC) cadets and midshipmen, and masters athletes. Maximal oxygen consumption ($VO_2$max), and resting metabolic rate (RMR) will be measured using mobile and stationary metabolic cart equipment, respectively. This equipment analyzes the volumes of oxygen inhaled and carbon dioxide exhaled to compute $VO_2$max and RMR. The determination of $VO_2$max will show the level of how fit a person is, and RMR will assess the Calorie needs of each participant. Levels of physical activity will be documented in collegiate athletes, ROTC cadets and midshipmen, and masters athletes through the use of accelerometers. These small devices will be worn

by the participants for one week, and they will detect the time, frequency, duration, and intensity of movement. Body composition traits, such as percent body fat, will be measured using bioelectrical impedance analysis (BIA) and dual-energy x-ray absorptiometry (DXA). Dietary intake will be evaluated using self-administered food frequency questionnaires that will allow us to get an idea of the participants' usual dietary intake. All of these factors ($VO_2$max, RMR, physical activity, body composition, and dietary intake) may have significant implications for physical performance. The researchers in this study also seek to analyze changes in these traits after a period of detraining or unsupervised conditioning. Therefore, you may be contacted for a second round of tests after your off-season.

### *7. How long will the research last?*

We expect that you will be in this research study for a period of one to two weeks depending on your availability. This one to two week period will include two testing appointments separated by approximately one week. You may be contacted for another round of testing after your off-season, but your participation will not be mandatory.

### *8. How many people will be studied?*

We expect more than 200 people will be in this research study.

### *9. What happens if I say yes, I want to be in this research?*

If you are willing to take part in this study, you will be required to complete an online survey, which will ask you about your exercise and lifestyle habits. From these data, we will determine if you qualify for the study. If you do qualify for the study, we will contact you via email to schedule your first laboratory appointment. Your first laboratory appointment will be in the morning and include a bioelectrical impedance analysis (BIA) test, a resting metabolic rate (RMR) test, and a dual-energy x-ray absorptiometry (DXA) scan. We will also schedule a $VO_2$max test for the following week at a late afternoon/early evening time.

**Session 1:**
You should allot 2.5 hours for this session. Upon your first arrival to the Department of Nutrition Sciences Laboratory, located at 1601 Cherry Street, Room 325A, you will undergo an RMR and BIA test. You will be asked if you adhered to the pre-testing requirements: 1) you kept a 12-hour overnight fast and 2) you refrained from caffeine for 10 to 12 hours as well as alcohol and exercise for 24 hours. You should be wearing comfortable clothing for the RMR test.
After verification of adherence to the pre-testing requirements, we will ask about your current medications, existing health conditions, and (females only) the date of the start of your last menstrual cycle. These factors have been shown to influence RMR.

**ROTC cadets and midshipmen only**: We are asking for your permission to have your physical fitness test results released to us for comparison to our laboratory measures of fitness. You do not have to consent to the release of this information from ROTC officials in order to be in this study. Please indicate your choice by initialing below:

_____ Yes, I agree to the release of my results.

Or

_____ No, I do not wish to allow access to my results.

Next, your height, body weight, and waist circumference will be measured. We will measure height and body weight twice on a calibrated scale. Waist circumference will be measured three times using a soft measuring tape. Multiple measurements will ensure accuracy. After recording these measurements, you will stand on the BIA instrument for body composition data collection. During a BIA test, an electrical current is transmitted through electrodes on the hands and feet. You cannot feel this electrical current, and the test is harmless. This instrument is used to estimate your percent body fat. Dehydration will lead to the overestimation of fat mass during BIA. Therefore, before a BIA appointment, participants will be asked to drink plenty of water that day.

You will then rest for 15 to 30 minutes in a reclining chair prior to RMR testing. After this rest period, a dilution hood (a clear plastic bubble) will be placed over your head to analyze inhaled and expired air. RMR testing will be completed using a Vmax™ Encore Metabolic Cart. This instrument measures your breathing, a non-invasive method that is of no risk to you. Data collection will take about 15 to 45 minutes. You will not be able to sleep, read, watch television or listen to music during RMR testing.

A dual-energy x-ray absorptiometry (DXA) scan will be conducted on the 2nd floor of the 3 Parkway building after your RMR test. The scanning device detects bone, fat, and muscle in the body. All female participants will be required to complete a urine pregnancy test before undergoing a DXA scan. A positive test will result in exclusion from the study. You should not wear clothes with metal parts during this test. You will lie flat on the bed of the scanner. The scan will take approximately 12 minutes. Your scan will be conducted by trained personnel, and a certified radiation technologist will be available to evaluate the results.

All of these testing procedures are expected to take approximately 2 hours to complete. However, you should allot 2.5 hours for your appointment. After completing this first appointment, a lab member will confirm your $VO_2$max test appointment time before you exit the lab. You will also receive your Actical™ accelerometer and instructions for its use . You will wear the Actical™ accelerometer on your wrist for 7 days. This device will measure your physical activity during this time period by detecting body movement. After 7 days, you will switch the device off and return it to a lab member when you return for your $VO_2$max testing session.

**Session 2:**

You should allot 2 hours for this session. Your $VO_2$max test will also be conducted at the Department of Nutrition Sciences Laboratory, located at 1601 Cherry Street, Room 325A. For your $VO_2$max test, it is important that you wear comfortable exercise clothing. We will use an Oxycon™ Mobile Device by CareFusion to analyze inhaled and exhaled air. A lab member will fit a facemask with a mouthpiece on your head. The mouthpiece will have a sensor that will be connected to the Oxycon Mobile Device equipment. The equipment will be strapped into a vest and carried on your back. You will also wear a chest-positioned heart rate monitor to measure heart rate during exercise testing. Before the test begins, you will be instructed on hand signals to give the experimenter to communicate your status or desire to end the test. These signals will avoid miscommunication or disruption of data collection.

Participants between the ages of 18 and 25 years of age will run at a speed of 7 mph for 2 minutes to warm up. Participants who are 26 years of age or older will run at a speed of 5.5 mph for 2 minutes to warm up. Afterward, the speed of the treadmill will remain at or increase to 7 mph and the incline of the treadmill will increase by 1% grade until you reach exhaustion. If an incline of 12% grade is attained, the incline will remain at 12%, and the speed of the treadmill will increase by 0.5 mph each minute until you indicate that you do not wish to continue. After you signal exhaustion, the speed will be reduced and the treadmill will return to a flat position for 3 minutes of cool-down. The test is expected to last 10 to 15 minutes.

After you have recovered from the $VO_2$max test, you will complete a food frequency questionnaire, which will take about 30 to 40 minutes to complete. You will bubble in your answers on the form with a no. 2 pencil. A portion size guide will be given with the questionnaire to help you in your selections. The questionnaire will evaluate your dietary patterns over the past year. Because you will complete the exercise test and the food frequency questionnaire at the same lab appointment, you should allot 2 hours for this entire testing session.

### *10. What happens if I do not want to be in this research?*

You may decide not to take part in the research and it will not be held against you.

### *11. What happens if I say yes, but I change my mind later?*

You agree to take part in the research now, and if you decide to stop at any time, it will not be held against you.

### *12. Is there any way being in this study could be bad for me?*

For RMR testing, you might feel hungry after the 12-hour fast; however, because testing will occur in the morning, you will be able to consume a meal after you leave the laboratory. You might feel uncomfortable sitting in the reclining chair under the dilution hood, but in the past, participants who have felt that way, relaxed after a few minutes. There are no other foreseen risks, discomforts, or constraints for RMR testing.

Participants will receive a very small radiation exposure from the DXA scan. The exposure is less than one might receive from natural background radiation in a single day. The risks, therefore, are correspondingly minimal to non-existent.

For $VO_2$max testing, it is important to note that there are some risks as with all other exercise tests. The American College of Sports Medicine states that overall risk of a cardiac event (e.g., heart attack, heart rhythm abnormalities, or death) during an exercise test is 6 events per 10,000 tests. However, this number applies to a mixed population, which includes high-risk cardiac patients. If you have a known cardiovascular condition, you should not participate in this study. We believe the risk will be lower for collegiate athletes, ROTC cadets and midshipmen, and masters athletes because of their level of physical activity and physical fitness.

### *13. Do I have to pay for anything while I am on this study?*

There is no cost to you for participating in this study.

### *14. Will being in this study help me in any way?*

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include learning how well your exercise program has worked for you. Participation in this study may help to increase scientific knowledge about the fitness of collegiate athletes, ROTC cadets and midshipmen, and masters athletes. Knowing the current $VO_2$max and percent body fat of the participants could help program leaders to determine if their physical training is ideal to reach optimal fitness. Coaches, trainers, and ROTC officials could potentially use this information to adjust physical conditioning programs, and thus improve performance of athletes and ROTC cadets and midshipmen. In addition, knowing Caloric needs and current dietary practices of athletes and ROTC cadets and midshipmen can help students and their organization leaders make diet plans to meet energy (Calorie) requirements. Without sufficient energy, muscle can be used as energy, which would have a negative effect on performance and overall health.

Measuring metabolic rate, $VO_2$max, and body composition in another setting would be costly to the participants; thus, you are also benefitting from having these tests conducted at no cost.

### *15. What happens to the information we collect?*

Efforts will be made to limit your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

### *16. Can I be removed from the research without my OK?*

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal include:

a) If all or part of the study is discontinued for any reason by the investigator or university authorities.
b) If you are a student, and participation in the study is adversely affecting your academic performance.
c) If you fail to adhere to requirements for participation established by the research team.

### *17. What else do I need to know?*

This research study is being done by Drexel University.

By signing this document, you are agreeing to allow a member of the research team to contact you in the future for follow up testing for this study. If we contact you for another round of testing, it is not mandatory that you participate.

Participation in this study is completely voluntary, and there is no monetary compensation. At the conclusion of the study, research personnel will send a data sheet with a summary of fitness variables to each participant.

# Authorization to Use and Disclose Protected Health Information

Federal law provides additional protections of your personal information that are described here.

## *A. Individually Identifiable Health Information That Will Be Collected*

The following personal health information about you will be collected and used during the research study and may be given out to others:

- Your name, date of birth height and weight;
- Information from physical exams and other tests or procedures described in this consent form.
- Information learned during telephone calls, surveys, questionnaires and office visits done as part of this research study;
- (ROTC cadets and midshipmen only) Results from your physical fitness test if you allow our access to this information

## *B. Who Will See and Use Your Health Information within Drexel University*

The researcher and other authorized individuals involved in the research study at Drexel University will see your health information during and may give out your health information during the research study. These include the researcher and the research staff, the institutional review board and their staff, legal counsel, research office and compliance staff, officers of the organization and other people who need to see the information in order to conduct the research study or make sure it is being done properly. Your health information may be disclosed or transmitted electronically.

## *C. Who Else May See and Use your Health Information*

Other persons and organizations outside of Drexel University may see and use your health information during this research study. These include:

- Governmental entities that have the right to see or review your health information, such as The Office for Human Research Protections, and the Food and Drug Administration.

If your health information is given to someone not required by law to keep it confidential, then that information may no longer be protected, and may be used or given out without your permission.

## *D. Why your health information will be used and given out*

Your information may also be used to meet the reporting requirements of governmental agencies.

## *E. If you do not want to give authorization to use your health information*

You do not have to give your authorization to use or give out your health information. However, if you do not give authorization, you cannot participate in this research study.

## F. How to cancel your authorization

At any time you may cancel your authorization to allow your health information to be used or given out by sending a written notice to Human Research Protection at 1601 Cherry Street, 3 Parkway Bldg., Mail Stop 10-444, Philadelphia, Pennsylvania, 19102. If you leave this research study, no new health information about you will be gathered after you leave. However, information gathered before that date may be used or given out if it is needed for the research study or any follow-up.

## G. When your authorization ends

Your authorization to use and give out your health information will end when the research study is finished.

## H. Your right to inspect your medical and research records

You will not be able to look at your research records while you are taking part in this research study. Your personal information will be made available in an emergency if doctors need this information to treat you. You can have access to your medical record and any research study information when the study is over. However, the researcher does not have to release research information to you if it is not part of your medical record.

## Permission to Take Part in a Human Research Study

### Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

**DO NOT SIGN THIS FORM AFTER THIS DATE →** 07/22/2017

| | |
|---|---|
| Signature of subject | Date |
| Printed name of subject | |
| Signature of person obtaining consent | Date |
| Printed name of person obtaining consent | |

**APPENDIX D ~ DATA COLLECTION SHEET**

# A Comparison of Fitness Characteristics in Collegiate Athletes, Reserve Officers' Training Corps (ROTC) Cadets and Midshipmen, and Masters Athletes

***For research personnel use only ~ does not leave the Lab**

**Date:** _____

Participant ID: _____

Food Frequency Questionnaire (FFQ) ID: _____

Number of days of exercise per week (from pre-screen): _____

Presently competes in chosen physical activity (circle one): YES NO

ROTC only ~ consent to Army Physical Fitness Test release: _____

Informed consent signed and witnessed (yes/no): _____

Date of Birth (month/date/year): _____

Age:_____ years

Females only ~ start date of last menstrual cycle: _____

Pregnancy test given by: _____

Pregnant (circle one): YES NO

Current medications: _____

Existing Conditions: _____

Currently on specific diet (medical or non-medical) (yes/no):

If yes, briefly explain: _____

Participant adherence to RMR pre-test protocol (abstained from alcohol and exercise for 24 hours; abstained from food and caffeine for 12 hours) (circle one): YES NO

Participated in any Nutrition Sciences Dept. (involving DXA) in last 6 months (circle one):

YES NO

## SESSION ONE

**Date:** _____

Clothing participant wore on test day: _____

Participant's self-defined water consumption on test day:

_____ (Be sure to write units; e.g., cups, Liters)

## Anthropometrics

Height (centimeters [cm]): _____ and _____

Average of heights measured: _____ cm

Average of heights measured: _____ inches (in)

(Note: 2.54 cm per inch)

Weight on scale (pounds [lbs]): _____ and _____

Average of weights measured: _____ lbs

Average of weights measured: _____ kilograms (kg)

(Note: 2.2 pounds per kg)

Waist Circumference (cm): _____ and _____ and _____

_____

Average of waist circumferences measured: _____ cm

Researcher(s) who collected anthropometric data:

_____

_____

**Date:** _____

## Bioelectrical Impendence Analysis (BIA)

Weight measured from BIA: _____ lbs

Weight converted from lbs to kg: _____ kg

Percent body fat from BIA: _____ %

Calculated lean body mass (LBM) from BIA: _____ lbs

[Note: (%Body Fat / 100) x wt in lbs = fat lbs ; Total wt lbs – fat lbs = LBM lbs]

Lean body mass converted from lbs to kg: _____kg

RMR measured from BIA: _____ kilocalories (kcal)/day

Intracellular water (ICW) content from BIA: _____ kg

Extracellular water (ECW) content from BIA: _____ kg

Total body water content (TWC) from BIA: _____ kg

Researcher(s) who conducted BIA: _____

## Accelerometry (using 1 minute Epochs; using first 7 days worn only)

Accelerometer number: _____

Accelerometer placement: _____

(Right or Left Wrist [need to use non-dominant hand], Waist or Ankle; but provide justification if waist band or ankle band is used):

---

Researcher(s) who gave accelerometer to participant:_____

**Date:** _____

## Resting Metabolic Rate (RMR)

Participant's self-defined hours of sleep before RMR test:

_____ Hours

Steady-state (SS) timetable

(mark time when participant first goes into SS, then any subsequent times in/out of SS):

If fan speed adjusted,

record time here:

| IN | OUT |
|---|---|
| First: | |
| | |
| | |
| | |
| | |
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| | |

| |
|---|
| |
| |
| |

RMR measured from Vmax metabolic cart

(15-minute steady state average): _____ kcal/day

Researcher(s) who measured RMR:

_____

_____

**Date:** _____

## Dual-Energy X-Ray Absorptiometry (DXA)

Percent body fat from DXA: _____ %

Lean Body Mass: _____ lbs

Lean Body Mass converted to kg: _____ kg

Fat Free Mass: _____ lbs

Fat Free Mass converted to kg: _____ kg

Total Body Bone Mineral Density (BMD): _____ grams/$cm^2$ ($g/cm^2$)

Lumbar (L2 to L4) BMD: _____ $g/cm^2$

Dual Femoral Neck BMD: _____ $g/cm^2$

Researcher(s) who performed DXA:

_____

_____

**SESSION TWO** **Date:** _____

Clothing participant wore on test day: _____

Participant's self-defined water consumption on test day:

_____ (Be sure to write units; e.g., cups, Liters)

Accelerometer turned in: YES NO

Researcher(s) who collected accelerometer: _____

Activity record collected: YES NO

Basic accelerometry data from participant after he/she wore accelerometer for one week:

Total kcal expenditure for length of wear: _____ kcals

Average total kilocalorie (kcal)/minute (min) expended: _____ kcal/min

Total sedentary minutes: _____ minutes

Average kcal/min in sedentary behavior: _____ kcal/min

Total light physical activity minutes: _____ minutes

Average kcal/min in light physical activity: _____ kcal/min

Total moderate physical activity minutes: _____ minutes

Average kcal/min in light physical activity: _____ kcal/min

Total vigorous physical activity minutes: _____ minutes

Average kcal/min in vigorous physical activity: _____ kcal/min

**Anthropometrics before Exercise Test**

Weight measured on scale: _____ lbs

Weight converted from lbs to kg: _____ kg

**Date:** _____

## Maximal Oxygen Consumption ($VO_2max$)

$VO_2max$ measured from Oxycon Mobile (estimate from 30-second average data):

_____ milliliters of oxygen/kg of body weight/minute (mL/kg/min)

Time reached $VO_2max$: _____ minutes

Researcher(s) who measured $VO_2max$:

_____

_____

## Food Frequency Questionnaire (FFQ)

Administered (yes/no): _____

Researcher(s) who administered FFQ:

_____

_____

**IF PARTICIPANT DROPPED FROM OR WAS ASKED TO LEAVE THE STUDY:**

Date of removal: _____

Reason for removal: _____

_____

_____

_____

_____

Researcher who completed removal of participant from the study:

Print Name: _____

Signature: _____

Date: _____

## APPENDIX E ~ ACTIVITY LOG

ACCELEROMETRY ACTIVITY LOG

**Note: Use this log if you marked any specific events with your accelerometer, then list what those respective events were**

Participant ID:_____ Date: _____

| DAY | ACTIVITY | TIME IN | TIME OUT |
|---|---|---|---|
| 1 | | | |
| 1 | | | |
| 1 | | | |
| 1 | | | |
| 2 | | | |
| 2 | | | |
| 2 | | | |
| 2 | | | |
| 3 | | | |
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